5.5 TERRESTRIAL ANIMALS

5.5.1 Small Mammals and Birds

5.5.1.1 Perchlorate Residues in Native Small Mammals and Birds

5.5.1.1.1 Introduction

Small mammals and birds are routinely used as sentinel species to assess environmental exposure to chemicals (movement of chemicals from the environment into organisms) and the possible effects resulting from any exposure. Their utility is often tied directly to their diet, relative small home ranges or foraging areas, and position in the food chain. For example, small mammals, such as most rodents, consume a variety of plant and animal matter, and rarely venture over an area much larger than a hectare. Birds also have a variable diet, and although they may venture over large areas during parts of their life, during the breeding season their home ranges are often quite restricted. Thus many small mammals and birds collected from a specific location can be viewed with confidence as living and feeding in the area of collection. In addition, their position near the bottom of most food chains makes them excellent conduits for chemical movement into top-level mammalian, reptile, and avian predators. The combination of these characteristics of small mammals and birds make them useful for monitoring exposure and effects to a variety of chemicals, including perchlorate.

Perchlorate, although a very water soluble compound, has been detected in a variety of matrices, including soil, water, and plants. Small mammals and birds routinely consume free standing water, plants, and the insects living in association with water, soil, and plants. Thus, the potential is high for small mammals and birds to consume some dietary component that contains perchlorate. However, potential for exposure does not always equate to actual exposure, although perchlorate has been detected in birds and small mammals at other sites contaminated with perchlorate (Smith et al., 2001). The purpose of this phase was two-fold. First, to assess actual exposure of small mammals and birds to perchlorate residues as a function of living near contaminated environments and consuming food and water from those contaminated sites. We hypothesized that small mammals and birds would harbor detectable concentrations of perchlorate as a function of living and feeding on contaminated areas. Results of this phase of the study were used to assess the degree of exposure that occurs in wildlife populations of animals and answer the basic question of whether perchlorate, a water-soluble compound, can accumulate to detectable levels in small vertebrates. Second, we wanted to assess the distribution of perchlorate among different tissues in a small mammal model given perchlorate under controlled conditions.

Perchlorate exerts its primary effect on animals by inhibiting uptake of iodide into the thyroid gland (Stanbury, 1952). This inhibition of iodide uptake in turn inhibits the production of thyroid hormones that are subsequently sent into the peripheral blood (Wolff, 1998). Therefore, analyzing the concentration of thyroid hormones in blood (specifically plasma) samples from animals is one effects measurement indicative of

exposure to perchlorate. We hypothesized that small mammals exposed to perchlorate would exhibit alterations (decreases) in thyroid hormone concentrations.

5.5.1.1.2 Methodology

5.5.1.1.2.1 Field Collections

Small mammals and birds were collected from one or more sites distributed throughout the Lakes Waco and Belton watersheds, as shown on **Figure 5-174**. All of these sites represented riparian areas or drainage ditches associated with suspected perchlorate-contaminated areas that drained into local creeks. These areas include the groundwater spring south of Oglesby Road (T18) (HCS), the spring fed tributary that feeds into Harris Creek (HCT), Harris Creek at Highway 84 West of McGregor (T19) (HC84), Station Creek at Highway 107 (T23) (SC107), the unnamed tributary near the waste water treatment plant at Highway 317 (T14) (TF), and S Creek at Highway 317 (T15) (NB317).

5.5.1.1.2.2 Animal Collections

A variety of passerine bird species (n = 12) and small mammals (n = 35) were collected from the locations near the NWIRP site near McGregor, Texas. Mist nets were placed along creeks and ditches, and monitored continuously while open and all captures euthanized and frozen at -20 °C in the field. Snap traps were set in similar locations to capture small mammals. Traps were set in the evening and checked the following morning, and all captures frozen at -20 °C in the field. Birds and rodents were placed in plastic bags, labeled with location, species, and date. Samples were transported back to TIEHH for analysis.

5.5.1.1.2.3 Water Collections

Water samples were collected into clean vials from just below the water surface wherever possible. All water samples (5 mL) were filtered and either analyzed for perchlorate ion directly, or diluted with distilled, deionized water and then analyzed. Soil samples were taken from the top 5 cm of soil. Soil samples were weighed, placed in glass jars, and extracted (mechanical agitation) with distilled, deionized water (2:1 water:soil). Water extracts were filtered and either analyzed for perchlorate ion directly, or diluted with distilled, deionized water and then analyzed. Vegetation, invertebrate, and vertebrate diet samples were collected from areas adjacent to where animals were collected. Samples were removed from soil, sediment, or water and placed in plastic bags. Prior to extraction, samples were air-dried, and weighed.

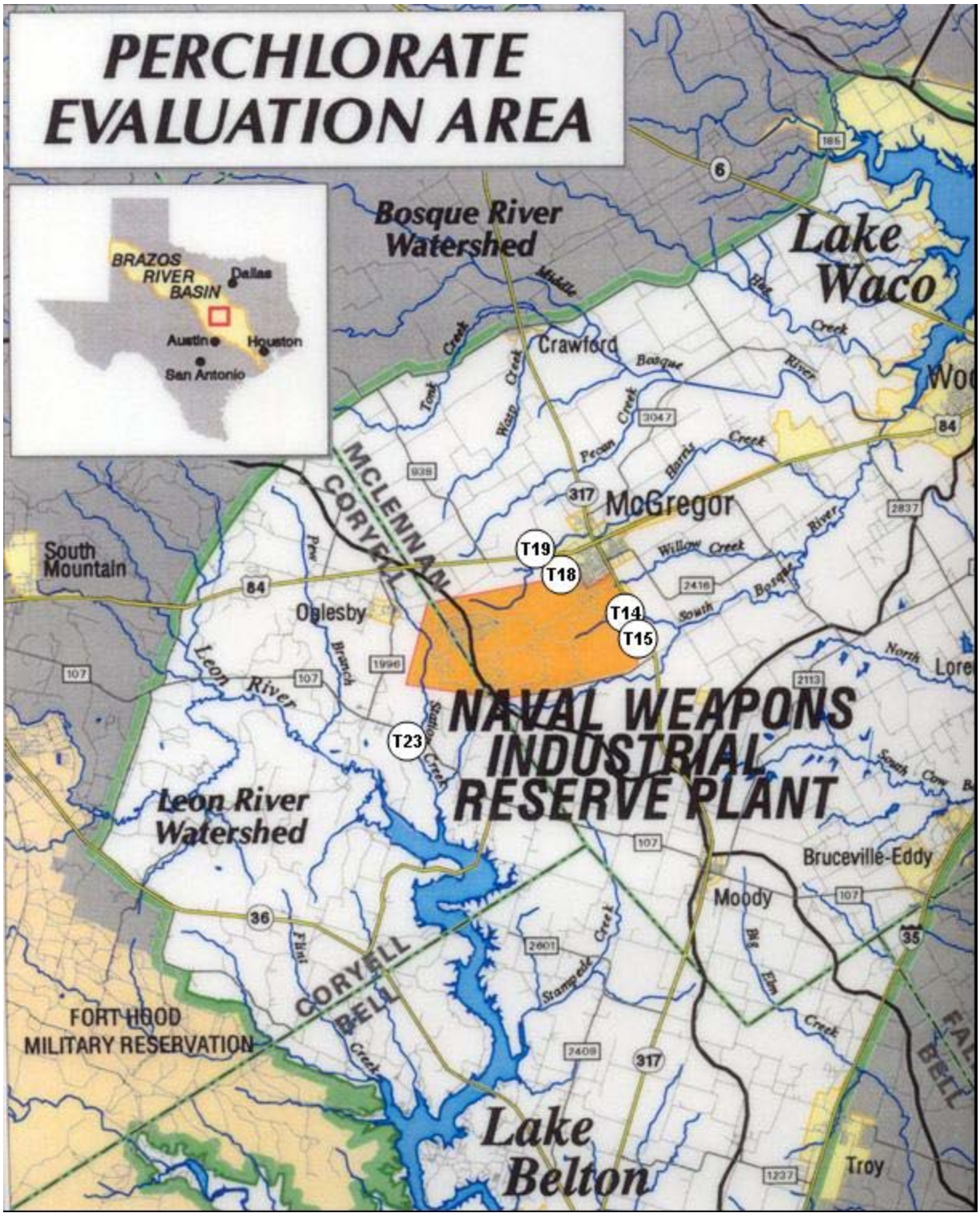

Figure 5-174
Map of Study Area Illustrating the Approximate Locations where Small Mammal and Bird Samples Were Collected

5.5.1.1.2.4 Tissue Distribution Study

Ten male voles were taken from our breeding colony housed at Texas Tech University for use in this study. These voles were randomly placed into 10 different metabolic cages (labeled 1-10) at 8:00 pm on September 1, 2003 with no food or water. Reference urine and feces were collected from the metabolic cages between 7:30 am and 8:00 am on September 2, 2003. At 8:00 am, upon completion of collection of reference materials, all voles were given 250 ppm magnesium perchlorate dissolved in water. The water bottles were weighed prior to putting them on the cages. The 10 voles were then randomly assigned to either a 4 hour or 8 hour dosing period. Vole 10, 6, 3, 5, and 9 were dosed from 8:00 am to 12:00pm. At the end of the 4 hours the water bottles were removed from the metabolic cages and weighed. These voles were euthanized using a carbon dioxide chamber and necropsied where blood, liver, kidney and thyroids were collected. Once all five voles were necropsied, the blood was spun down to collect plasma. All samples were stored on dry ice until returning to The Institute of Environmental and Human Health where they were stored in a -80 °C freezer until analysis. Urine and feces were collected from the cages upon completion of the necropsy. Vole 4, 7, 1, 2, and 8 were dosed from 8:00 am to 4:00 pm and then processed as described above for the 4 hour group.

5.5.1.1.2.5 Sample Analysis

Livers and kidneys were analyzed for perchlorate content using standard tissue extraction and analysis techniques developed in the analytical core of this project (**Appendix X**).

5.5.1.1.2.6 Statistical Analysis

Chi-square analysis was used to compare frequency distributions of quantifiable versus pooled trace and non-detectable concentrations of perchlorate in small mammals across sites and species. Linear regression was used to compare the relationship between concentrations of perchlorate in rodent tissues and water from collection sites. Two-tailed T-tests were conducted to test differences in water consumption, urine production, and perchlorate recovery in voles in the tissue distribution study. All summary statistics are reported as means and standard errors.

5.5.1.1.2.7 Detection Limits

All positive indications of perchlorate concentrations below the detection limits (**Table 5-54**), below the 2.5 ppb standard's area, or with less than a 2:1 signal to noise ratio were reported as trace amounts. A non-detect (ND) indicates that no positive indication of perchlorate was found in the sample. Minimum detection limit concentrations were estimated considering the chromatograph minimal detection area for each set of standards in conjunction with conservative extract volume and sample weight values of similar sample types. The samples were divided into categories based on tissue type and animal class, for example: mammalian kidneys and avian livers were two of the categories examined. Based on this examination and estimation minimum detection limits were determined.

Table 5-54
Minimum Detection Limits of Perchlorate in Mammal and Avian Tissues

Mammalian Kidney	5 ppm
Mammalian Liver	3 ppm
Avian Kidney	8 ppm
Avian Liver	6 ppm

5.5.1.1.3 Data

5.5.1.1.3.1 Field Collections

A total of 35 small mammals and 12 birds were collected (**Table 5-55**). Most small mammals and birds were collected from the Harris Creek site (HC84) and an adjacent site, HCT. Small numbers of mice and one bird were collected from the remaining sites. The lack of captures was due to sparse habitat at most sites and a concomitant lack of animals.

Table 5-55
Number of Individual Small Mammals and Birds Captured at the Six Field Site Locations in the Waco/Belton Watershed Near McGregor, Texas

Species	HC84 (T19)	HCT (T18-T19)	TF (T14)	NB317 (T15)	HCS (T18)	SC107 (T23)
Small Mammals						
House mouse	6	0	0	0	0	0
Peromyscus sp.	3	1	3	2	2	4
Harvest mouse	1	0	0	0	0	0
Cotton rat	13	0	0	0	0	0
Birds						
Eastern phoebe	0	3	0	0	0	0
Lincolns sparrow	0	1	0	0	0	0
Mockingbird	0	1	0	0	0	0
Northern cardinal	0	3	0	1	0	0
Song sparrow	0	1	0	0	0	0
White-crowned sparrow	0	2	0	0	0	0

1 See methods for descriptions of field site abbreviations.

Perchlorate was detected in kidney and liver samples from small mammals and birds (**Table 5-56**). In general, perchlorate was detected in quantifiable amounts more often in kidney than liver tissues. Quantifiable concentrations ranged from 4 ppm to 64 ppm in kidney samples and 7 ppm to 40 ppm in liver samples in small mammals. Frequency distributions of quantifiable concentrations of perchlorate in small mammals differed from trace/non-detectable perchlorate residues in both kidney and liver samples (kidney: $X_2 = 10.9$, d.f. = 1, $P < 0.005$; Liver: $X_2 = 5.3$, d.f. = 1, $P < 0.025$). This analysis compared frequency distributions among four sites; HC84, HCS and HCT combined, SC107, and TF and NB317 combined. Sites were combined based on proximity to one

another and existing in common drainages. Results for perchlorate in kidney indicated that quantifiable perchlorate was detected a less than expected number of times in mice on site HC84, and higher than expected on site SC107. Results differed for liver samples, with a higher than expected number of quantifiable detections of perchlorate in mice from HCS/HCT and TF/NB317, and a lower than expected number in mice from HC84.

Table 5-56
Perchlorate Concentrations in Tissues from Small Mammals and Birds Collected from Riparian Areas in the Waco/Belton Watershed Near McGregor, Texas

Biota	Site	Common Name	Kidney		Liver	
			Perchlorate (ppm)	Dry Wt. (g)	Perchlorate (ppm)	Dry Wt. (g)
Mammals	HC84 (T19)	House mouse	Trace*	0.1026	ND	0.3849
			Trace*	0.0569	ND	0.2248
			Trace*	0.0604	ND	0.2408
			Trace*	0.0542	ND	0.2549
			Trace*	0.0711	11	0.2732
			27*	0.0835	ND	0.3928
		Deer mouse	Trace*	0.0989	ND	0.3654
			Trace*	0.1046	ND	0.4735
			Trace*	0.0703	ND	0.3288
		Harvest mouse	Trace*	0.0462	ND	0.1613
		Cotton Rat	Trace*	0.1778	Trace*	1.1635
			4*	0.1276	7*	0.6775
			10*	0.2002	ND*	1.0321
			8*	0.2949	ND*	1.6693
			17*	0.0581	Trace*	0.2386
			Trace*	0.2243	10*	1.1098
			Trace*	0.3485	Trace*	1.9194
			Trace*	0.1738	Trace*	0.9738
			Trace*	0.164	ND*	0.7288
			Trace*	0.1503	Trace*	0.7989
			Trace*	0.264	Trace*	0.9692
			Trace*	0.3008	Trace	2.0421
			Trace*	0.2463	Trace	1.215
	HCS (T18)	Deer mouse	38*	0.0732	ND	0.2436
			Trace*	0.0809	14	0.2484
	HCT (T18-T19)	Deer mouse	26*	0.0898	40	0.3359
	SC107 (T23)	Deer mouse	23*	0.0809	12	0.2668
			7*	0.1042	ND	0.4732
			28*	0.1055	Trace	0.3768
			34*	0.0918	ND	0.3629
	TF (T14)	Deer mouse	28*	0.0757	10	0.3396
			64*	0.0773	Trace	0.1809
			45*	0.0928	ND	0.4306
	NB 317 (T15)	Deer mouse	ND	0.0539	17	0.2317

Biota	Site	Common Name	Kidney		Liver	
			Perchlorate (ppm)	Dry Wt. (g)	Perchlorate (ppm)	Dry Wt. (g)
			ND	0.0452	ND	0.2411
Birds	HCT (T18-T19)	Eastern Phoebe	86*	0.0457	Trace	0.1575
			28*	0.0509	ND	0.1205
			17*	0.1395	9	0.3905
		Lincoln's Sparrow	37*	0.037	ND	0.1747
		Mockingbird	18*	0.156	ND	0.9404
		Northern Cardinal	10*	0.119	7	0.4701
			31*	0.137	ND	0.3474
			17*	0.0921	ND	0.3545
		Song Sparrow	32	0.1127	47	0.2659
		White Crowned Sparrow	42*	0.0813	ND	0.3441
			19*	0.0751	7	0.4132
	NB 317 (T15)	Northern Cardinal	ND	0.1023	ND	0.3691

* indicates extraction with EtOH instead of water

Mean concentrations of perchlorate in kidney samples of rodents were also compared to mean concentrations of perchlorate in water samples collected from the different sampling areas (**Figure 5-175**). Variation in perchlorate in water samples explained 27% of the variation in perchlorate concentrations in kidney samples across four sampling areas.

Frequency distributions of quantifiable perchlorate concentrations in tissues also differed among rodent species pooled across sites. House mice, deer mice, and cotton rats, but not harvest mice (n = 1) were compared to assess the distribution of quantifiable versus trace and non-detectable perchlorate in kidney and liver samples. The frequency of quantifiable perchlorate differed across species in kidney samples (X^2 = 4.3, d.f . = 1, P < 0.05) but not for liver samples (X^2 = 1.5, d.f. = 1; P > 0.100). The number of quantifiable perchlorate concentrations in kidney samples was higher than expected in deer mice.

These same analyses could not be performed for birds given the low number of captures across sites (all but one bird was captured at HCT). However, perchlorate concentrations in birds at HCT tend to agree with the elevated concentrations of perchlorate found in small mammals from the same site or same general area (e.g., HC84, HCS, and HCT).

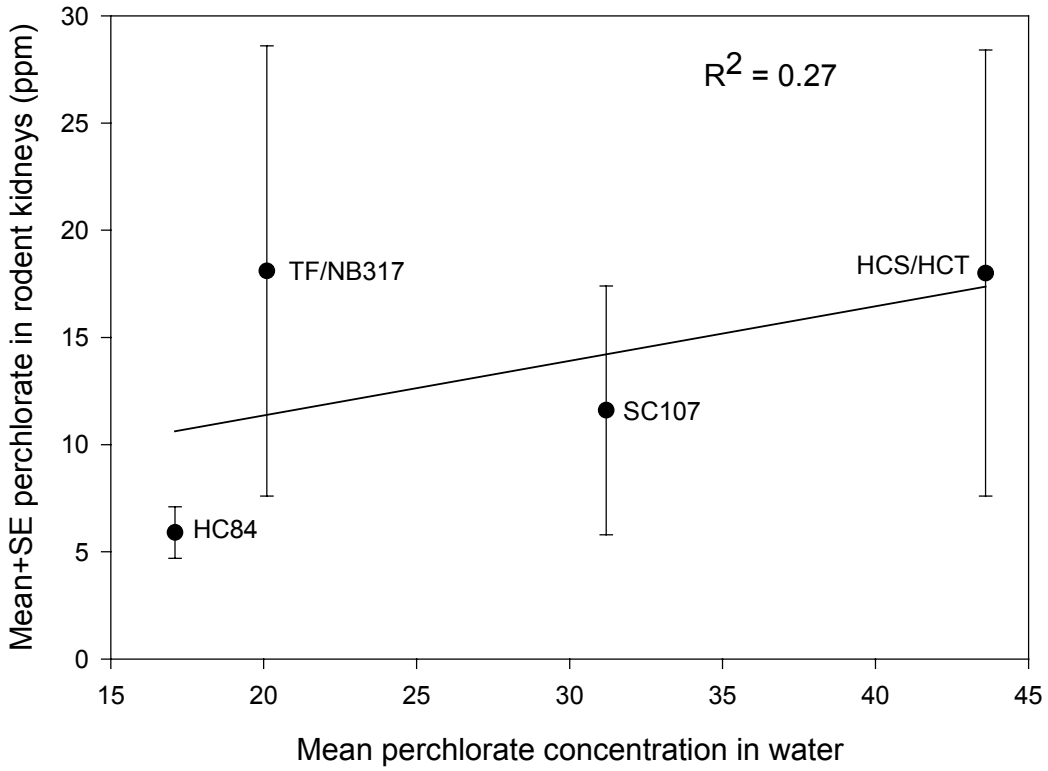

Figure 5-175
Linear Regression of Perchlorate Concentrations in Rodent Kidneys Versus Perchlorate Concentration in Water Samples from Study Sites in the Lake Waco and Lake Belton Watersheds near McGregor, TX

5.5.1.1.3.2 HC84 (T19)

Peromyscus spp. (deer mice or white-footed mice), and one harvest mouse contained trace concentrations or concentrations below our minimum detection limit (ND). However, two house mice and five cotton rats contained elevated perchlorate concentrations in kidneys and/or livers. Yet individuals of both species were also collected that contained no quantifiable perchlorate residues. Interestingly, higher concentrations were detected in kidneys than livers in general, perhaps indicating a recent or intermittent exposure since kidneys remove, store, and eliminate ions from blood so as to maintain osmotic balance. Quantifiable kidney concentrations ranged from 4-17 ppm and 7-10 ppm in liver samples.

5.5.1.1.3.3 HCS (T18)

Two *Peromyscus* were collected at this location, both containing elevated tissue concentrations of perchlorate. Few rodents were collected at this site despite extensive sampling effort. This site provides surface water year-round, potentially serving as a source of exposure to birds and mammals even during drought periods.

5.5.1.1.3.4 HCT (Between T18 and T19)

This site offered opportunities to erect and monitor mist nets intended for bird capture and collection. Among the avian species collected were migratory and part-time resident species. All birds collected from this area contained elevated perchlorate concentrations in kidneys, and many contained quantifiable concentrations of perchlorate in liver tissues. Highest tissue concentrations were found in the kidneys of an Eastern phoebe (86 ppm) and a liver sample from a Song sparrow (47 ppm). These tissue concentrations exceed the highest ever reported for any wildlife species (see Smith et al., 2001). Currently, there are no data available to evaluate potential effects related to these tissue concentrations. One *Peromyscus* collected from this area also contained elevated kidney and liver concentrations.

5.5.1.1.3.5 SC107 (T23) and TF (T14)

Four *Peromyscus* were collected from SC107 and three from TF, all of which contained elevated tissue perchlorate residues. As seen in other rodents and birds collected during this sampling period, perchlorate was detected more frequently in kidneys than livers. TF produced rodents containing the highest tissue concentrations among all rodents collected. One *Peromyscus* contained 65 ppm perchlorate in kidneys, again considerably higher than values reported in the scientific literature.

5.5.1.1.3.6 NB317 (T15)

Two deer mice and one Northern cardinal were collected from NB317. All but one of the tissues from these animals were non-detectable for perchlorate. One sample, a liver from a deer mouse had 17 ppm perchlorate. This value generally agrees with other perchlorate concentrations in liver samples from rodents in this study.

5.5.1.1.3.7 Tissue Distribution Study

Ten voles were used in this study, although one vole was excluded from analysis due to exceedingly high intake of water (10.31 mL) that was about two-fold greater than the means for either group (**Table 5-57**). The remaining nine voles were used to calculate summary statistics and test differences between means.

Table 5-57
Mean ± SE Values for Water Consumption, Urine Production, and Perchlorate Recovery in Voles Dosed with 250 ppm Perchlorate in Drinking Water for Either 4 Hours or 8 Hours Following a 12 Hour Fasting Period

Group	Water Consumption (mL)	Urine Volume (mL)	Percent Perchlorate Recovered			
			Urine	Blood	Kidney	Liver
4 hour	4.8 ± 0.6	1.3 ± 0.5	33 ± 12	2.0 ± 0.4	0.03 ± 0.02	0.19 ± 0.10
8 hour	5.6 ± 0.3	2.6 ± 0.2	86 ± 7	1.6 ± 0.2	0.02 ± 0.01	0.22 ± 0.05

Water consumption did not differ between the 4 and 8 hour groups (P = 0.8190). In contrast, urine production was two-fold greater in the 8 hour group than in the 4 hour group. This difference was not statistically significant (P = 0.0653), but may indicate

biological significance. The lack of statistical significance in this test was undoubtedly driven in part by low sample size.

Most of the perchlorate was accounted for in the urine, with 33% and 86% recovered from voles in the 4 hour and 8 hour groups, respectively (**Table 5-57**). Likewise, recovery of perchlorate differed between groups ($P = 0.0149$). Conversely, little perchlorate was recovered in either blood, kidney, or liver samples in either group. Also, perchlorate recoveries did not differ between groups for any of these matrices ($P > 0.400$).

As expected, most of the perchlorate consumed by the voles was expelled in the urine. This was especially evident in the 8 hour group. Little perchlorate was observed in blood, kidney, or liver samples, indicating that these tissues are not efficient sinks for perchlorate under the constraints of this study. Of interest was our inability to account for all of the perchlorate in the four matrices examined, especially in the 4 hour dose group. This may be a function of time of exposure, with voles in the 4 hour group experiencing a latent period where much of the consumed perchlorate still resides in the intestinal tract. Voles in the 8 hour dose group have likely had more time to equilibrate to the exposure, with more of the consumed perchlorate passing out of the intestinal tract, into circulation, then passed in the urine. This idea is supported by the lack of differences in water consumption in conjunction with a two-fold increase in urine output in the 8 hour versus the 4 hour group.

The results of this study confirm previous work demonstrating that large portions of ingested perchlorate are expelled in the urine. However, we have observed high concentrations of perchlorate in kidney samples, and to a lesser degree, liver samples, in small mammals and birds. The short-term exposure and recovery period for perchlorate in this study does not mimic the potential long-term and continuous exposure experienced by wildlife in the field. Long exposure periods may result in accumulation not seen under laboratory conditions.

5.5.1.1.4 Discussion

These data should be interpreted with caution due to small sample sizes (both in terms of numbers and physical dimension), sampling design (collections from areas of known contamination only), reporting of dry weight tissue concentrations, and a lack of reference samples collected from non-impacted areas. Nevertheless, these data demonstrate significant exposure among migratory avian species and rodents inhabiting areas containing impacted waterways.

The rodent data suggest that perchlorate concentrations in tissues follow a gradient of potential exposure, with concentrations increasing in tissues with increasing concentrations in water. These data are weak however in that sample sizes were low for all sites except HC84. A more robust data set would add to the resolution in the relationship between environmental concentrations and tissue concentrations. Nonetheless, the evidence supports differential exposure among sites in terms of concentration gradients and frequency of quantifiable perchlorate in rodents.

Different species of wildlife vary in their inherent life-history traits. Differences in foraging strategies (e.g., diet, food processing), habitat preferences, home ranges, and others all play a role in how an individual species interacts with its environment, and thus dictate the exposure scenario in a contaminated environment. Observed differences among rodent species in the frequency distribution of quantifiable concentrations of perchlorate are not overtly surprising. House mice, deer mice, and cotton rats, although occupying the same general habitats, can differ in diet composition and how they process forage. Species in the genus *Peromyscus* (e.g., deer mice, white-footed mice) often consume diets consisting of relatively high percentages of invertebrates, although diets do vary tremendously (Lackey et al., 1985). By comparison, cotton rats typically consume a high percentage of plant material, particularly monocots (grasses; Kincaid and Cameron, 1982). House mice often have omnivorous diets, consuming a variety of plant and invertebrate material (Miller and Webb, 2000). However, these apparent differences must be viewed with caution as diets of many small mammals are notoriously flexible and dependent on availability of the different diet components. The differences observed in the frequency of quantifiable concentrations of perchlorate among small species in this study may be directly attributable to differences in diet composition and food processing techniques.

Similar variation among species would likely occur in birds as well, with perchlorate exposure varying among species depending on diet. Birds such as northern cardinals and different species of sparrows consume diets dominated by seeds, whereas mockingbirds and phoebes consume copious amounts of invertebrates. Unfortunately the bird collections in this study were essentially restricted to site HC84, minimizing our overall sample size. At site HC84, perchlorate concentrations in kidneys of insectivores (phoebes and mocking birds; 37.3 ± 16.4 ppm) did not differ from granivorous birds (cardinals and sparrows; 26.9 ± 4.4 ppm; $P = 0.5794$). However, the trend toward higher concentrations in insectivorous birds is evident.

Results from small mammals and birds in this study demonstrate that wildlife receptors are being exposed to perchlorate, that exposure may differ among species as a function of their diet, and that exposure varies across sites, possibly in a dose-dependent manner.

5.5.1.2 Thyroid Hormones in Native Small Mammals and Birds

5.5.1.2.1 Introduction

Perchlorate exerts its primary effect on animals by inhibiting uptake of iodide into the thyroid gland (Stanbury, 1952). This inhibition of iodide uptake in turn inhibits the production of thyroid hormones that are subsequently sent into the peripheral blood (Wolff, 1998). Therefore, analyzing the concentration of thyroid hormones in blood (specifically plasma) samples from native animals is one effects measurement indicative of exposure to perchlorate. We hypothesized that small mammals exposed to perchlorate would exhibit alterations (decreases) in thyroid hormone concentrations.

5.5.1.2.2 Methodology

Hormone analysis was not conducted in field captured mice and rats. Rodents captured in the field were collected with snap-traps to minimize or eliminate depuration of perchlorate from the body. This trapping method precludes collection of plasma. Therefore, in order to estimate the effects of perchlorate on native rodents, laboratory studies were conducted on deer mice (*Peromyscus maniculatus*) and prairie voles (*Microtus ochrogaster*).

Thirty nine adult male deer mice were dosed with perchlorate in food and drinking water for 44 days. Concentration of perchlorate was 5.3 ppm in food and 2.2 ppm in water, with 0 ppm controls. Mice were terminated after 44 days and plasma samples collected and stored frozen until analysis of thyroid hormones.

One hundred and twenty adult prairie voles were dosed with perchlorate in food and drinking water for 21 to 63 days. Concentration of perchlorate was 2.1 ppm in food and 0.99 ppm in water, with 0 ppm controls. At the end of each exposure period, voles were terminated and plasma and tissue samples collected for hormone, residue, and histological analyses. Plasma and tissues for residues were stored frozen until analysis. Tissues collected for histological analysis were stored in fixative until sectioned and examined under a light microscope.

Thyroid hormone analysis was conducted using Diagnostic Products Coat-A-Count Total kits (TKT31 and TKT45 respectively; Diagnostic Product Corporation, Los Angeles, CA). If plasma volume was insufficient for both assays, T_3 was run first, followed by T_4. When there was not a sufficient volume of plasma to run T_3 analysis (250 µL), only T_4 analysis (70 µL) was performed. The kits detected both free and protein-bound hormone (T_3 and T_4). Total T_3 and T_4 concentrations were calculated from the relative binding of a known amount of radio-labeled hormone to the antibodies on the tube and the binding of the unknown amount of hormone in the sample. Radioimmunoassays were conducted using standard procedures described in the manufacturers' instructions. RIA test kits used for hormone analysis were optimized for detection of T_3 and T_4 concentrations in the rodent plasma.

Thyroids from all voles were fixed in a triple aldehyde electron microscopy fixative (Tandler, 1990) for 10 minutes and preserved in formalin for histological processing. A Tissue-Tek V.I.P. 2000 Processor (Miles Scientific, Naperville, IL) was used to process tissues which were then embedded in paraffin. Paraffin blocks containing thyroid tissue were cut into sections at 7 µm with a microtome (Cut 4055, Olympus America Inc., Melville, NY). Ribbons of tissue sections were then mounted on glass microscope slides and stained following a basic hematoxylin and Eosin Y staining technique (Hinton, 1990).

Slides were examined with an Olympus BX52 compound light microscope and photographed with an attached Olympus DP1-L digital camera (Olympus Optical Co., Ltd, Tokyo, Japan) at 40X magnification. A section from the beginning, the middle, and the end of each specimen was selected from slides containing thyroid tissue to assess

overall histological status of each thyroid. Thyroid tissue from both lobes (right and left) of the thyroid was examined. Thyroid tissue was examined and blindly scored for the presence and severity of hyperplasia, hypertrophy, and colloid depletion based on guidelines established by the Pathology Working Group (Mann, 2000). Each trait was given a score of zero (0) for no indication of damage, 1 for slight damage (minimal), or 2 for severely damaged (severe) (Mann, 2000).

5.5.1.2.3 Data

Deer mice. Concentrations of thyroid hormones varied among treatment groups. Mean concentration of T_4 was approximately 50% greater in mice dosed via food than control mice ($P = 0.0108$). However, mice dosed via water showed similar T_4 concentrations as control mice. T_3 concentrations did not differ among treatment groups ($P > 0.05$).

Prairie voles. Concentrations of perchlorate in liver and kidney were determined in 150 adult voles. Only six voles showed detectable concentrations in liver samples, ranging from 0.183 ppm to 1.9 ppm. Likewise, perchlorate was detected in only five kidney samples, ranging from 1.0 ppm to 5.6 ppm.

Thyroid hormone concentrations varied little among treatment groups (**Table 5-58**). Concentrations of T_3 did not differ among treatments ($P = 0.7133$); likewise, T_4 concentrations were similar among treatments ($P = 0.3700$).

Table 5-58
Mean (± SE) Concentrations for T_3 and T_4 Hormones in Adult Prairie Voles Dosed with Perchlorate in Food and Water, and Controls

	n	Thyroid Hormone Concentration T_3 (µg/dL)	n	Thyroid Hormone Concentration T_4 (µg/dL)
Control	29	104.4 ± 8.9	27	2.3 ± 0.2
Food	42	108.9 ± 5.0	40	2.5 ± 0.2
Water	43	102.0 ± 5.7	35	2.6 ± 0.2

Histological examination of the thyroid gland of exposed and non-exposed voles was used to augment hormone data, as histological lesions are often considered a less transient effect of exposure to perchlorate. Thyroid glands of 102 adult voles were examined for follicular cell hyperplasia/hypertrophy and follicular cell colloid depletion (**Table 5-59**).

Table 5-59
Occurrence of Follicular Cell Hyperplasia/Hypertrophy and Follicular Cell Colloid Depletion (Incidence/Number of Thyroid Glands Examined) in Prairie Voles Dosed with Perchlorate in Water, Food, and Controls

Endpoint		Male			Female		
		Control	Water	Food	Control	Water	Food
Hyperplasia/ Hypertrophy	Minimal	0/15	2/19	1/19	0/18	2/15	0/16
	Severe	0/15	0/19	0/19	0/18	0/15	0/16
	Total	0/15	2/19	0/19	0/18	2/15	0/16
Colloid depletion	Minimal	4/15	8/19	4/19	3/18	8/15	2/16
	Severe	0/15	0/19	0/19	0/18	0/15	0/16
	Total	4/15	8/19	4/19	3/18	8/15	2/16

(See methods for description of Scores (minimal and severe)

Hyperplasia of the follicular cells was observed concurrent with hypertrophy. These alterations were observed only in the voles dosed with perchlorate, but at a low frequency (7.9% of the dosed animals). Conversely, colloid depletion of follicular cells was observed in all groups, including controls, and at a higher rate than for hyperplasia and hypertrophy (**Table 5-59**). Colloid depletion was observed at two to four times greater incidence in voles exposed to perchlorate in water than food exposed or control voles (X^2 = 8.66, d.f .= 2, P < 0.025).

5.5.1.2.4 Discussion

Results from native rodents indicate that exposure to perchlorate resulted in alterations in either thyroid hormones or thyroid structural pathology. However, differences did exist between species, with deer mice exhibiting altered hormone concentrations, which was not observed in voles. But in voles, thyroid histology did show alterations even in the absence of hormone alterations. Dosing designs differed between the two species, with deer mice exposed to higher concentrations of perchlorate for a consistently longer period of time. This higher level of exposure overall could account for the observed differences in thyroid hormones that was not observed in voles. Deer mice, although not showing hormone alterations, do exhibit classic signs of perchlorate effects with an increased incidence of colloid depletion in follicular cells. This result could indicate that although perchlorate is acting on the target cells in the thyroid, effects on actual hormone production have not been reached and/or animals are still operating on reserve hormones stored in the thyroid.

In comparison to field captured rodents, the laboratory dosed animals appear to show much lower exposure. This is evidenced by the low, and rarely observed, concentrations of perchlorate in either liver or kidney samples from voles. It is difficult to estimate the daily exposure of perchlorate to wild rodents, or the duration and frequency of exposure in the field. However, given the known doses in the laboratory studies and subsequent tissue burdens, it is safe to assume that rodents captured in the field are easily exposed to equal or greater amounts of perchlorate, as they routinely show parts per million levels of perchlorate. Given this observation and the laboratory results (Thuett et al., 2002), we

estimate that the field exposed animals would likely show alterations in hormone concentrations and/or thyroid histology.

The exact ramification of these lesions is difficult to determine. Recent work in our laboratories show that deer mice and/or voles exposed to perchlorate at environmentally relevant doses show alterations in metabolic activity and/or reproduction. Metabolic activity increased, and then began decreasing over a 30 to 40 day exposure period in deer mice exposed to perchlorate in water and food. These results suggest that continued exposure to perchlorate at environmentally relevant doses could alter metabolic function, leading to alterations in energy regulation and thermogenesis. These alterations in normal homeostasis may have significant effects on animals during periods of growth, reproduction, and varying climate extremes.

5.5.1.3 Pharmacokinetic/Population Modeling of Small Mammals and Birds

5.5.1.3.1 Introduction

A mathematical model was developed and computer simulations were conducted of the effects of perchlorate on populations of small mammals in the Bosque and Leon Rivers Watersheds in Central Texas. Compartments in the small mammal model include blood plasma, liver, kidneys, gut wall, gut contents, and the thyroid gland. The primary exposure pathway in small mammals is ingestion of food and water contaminated with perchlorate. Random variables include perchlorate concentrations in food and water. Using a time-step of one hour, the model currently runs simulations of one year (8760 hours) for each individual animal. Several individuals are simulated to obtain mean and 95% confidence limits on perchlorate concentrations in each compartment.

A second mathematical model was developed and computer simulations were conducted of the effects of perchlorate on populations of Bobwhite quail in the Bosque and Leon Rivers Watersheds in Central Texas. The modeling objectives were to (1) predict the uptake and distribution of perchlorate in avian body tissues, (2) predict maternal transfer of perchlorate to eggs, and (3) predict the effects of ammonium perchlorate on the thyroid hormone system. The model contains random variables for the concentrations of perchlorate in drinking water and in other dietary components, as well as the number of eggs produced by female quail.

5.5.1.3.2 Methodology

5.5.1.3.2.1 Model Testing

The modeling approach was to develop and apply mathematical models, either through modification of off-the-shelf models or de novo development, as appropriate, and parameterize the models using data from laboratory studies above.

Software tests are intended to challenge the application software and other parts of the overall system functionally and structurally. Functional testing demonstrates only that the system outputs appear to be correct. It does not allow an assessment of whether the software is actually performing according to specifications and requirements. A complete

functional test of every combination of inputs may not be feasible except for very small programs. Functional testing is essentially a subset of structural testing.

Structural testing is designed to exercise all modules and branches of the software and their interrelationships with the hardware and peripheral devices. Structural testing is performed to ensure that all relevant functions in the software perform as intended.

Each of the testing types described below was conducted. Performing only one type of test will not prove that the system is working properly.

Normal testing includes cases that test the functional and structural integrity of the computerized system. The input data for these test cases all fall within the range the user considers to be normal. Performing enough test cases can give a reasonable level of confidence that the system behaves as intended under normal conditions.

Boundary testing is performed using values that force the system to discern whether the input is valid or invalid, or to make a decision as to which branch of the program to execute. Boundary test values are set at the edges (i.e., slightly below and above) of valid input ranges. Boundary testing does not mean making the computerized system "crash" or involuntarily stop.

Special Case testing, also known as "exceptional case testing", documents the system's reactions to specific types of data or lack of data and is intended to ensure that the computerized system does not accept unsuitable data. These tests should be designed to document what happens when values that are not included in the ranges defined in the specifications are entered. Use of test cases with no data entry in a field will assist in establishing software system defaults.

Parallel testing is one of the most common types of tests performed by software developers. Parallel testing is performed by running two systems in parallel and comparing the outputs (e.g., two software application versions or software compared with a manual procedure). The comparison of the outputs from the same software release on different systems or different releases on the same system is part of parallel testing plans. Parallel testing can be a valuable tool when it is used in conjunction with other testing types for validation, or to train personnel to use a new computer system.

The model was tested with each of the structural tests described above and passed each test.

5.5.1.3.2.2 Model Development

A mathematical model was developed and computer simulations were conducted of the effects of perchlorate on populations of small mammals and bird species in the Bosque and Leon watersheds near Waco, Texas. The model consists of two sub-models: (1) a physiologically based toxicokinetics (PBTK) model of the uptake and distribution of perchlorate in mammal and bird body tissues for each individual in the population, including maternal transfer of perchlorate from mother to egg, and (2) a model of the

thyroid hormone secretion as affected by the perchlorate concentration at the thyroid. The model is stochastic in that it contains random variables for the concentrations of perchlorate in drinking water and in other dietary components, as well as the number of eggs produced by female quail. These random variables provide the capability to conduct Monte Carlo simulations.

5.5.1.3.2.3 Model Description

The PBTK model includes compartments for blood plasma, kidney, liver, skin and fat, thyroid, heart, gut contents, gut wall, and egg (**Figure 5-176**).

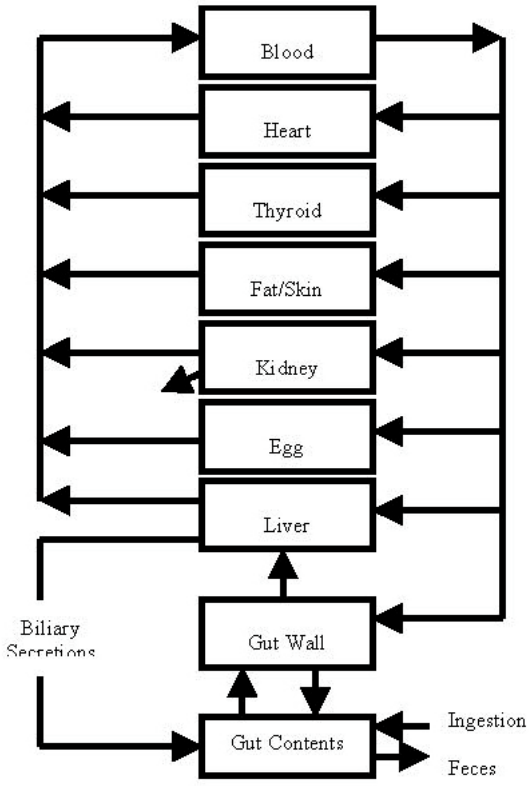

Figure 5-176
Flow Diagram of Ammonium Perchlorate PBTK Model

The organ compartment concentrations of perchlorate in an individual between time *t* and time *t+1* results from the rates of ingestion and elimination of perchlorate in the interval. The dose at time *t+1*, Q_{t+1}, then can be described by the general difference equation (1):

$$Q_{t+1} = Q_t - \left(\frac{F \bullet (Q_t / v)}{P} \right) + \left(\frac{F \bullet (Qplasma_t / v)}{P} \right) \qquad (1)$$

where,

Q_t = perchlorate compartment burden at time *t*, mg • L $^{-1}$

Q_{t+1} = perchlorate compartment burden at time $t+1$, mg • L $^{-1}$
F = Blood flow rate into the compartment, L/hour
v = Volume of compartment, L
P= Compartmental partitioning coefficient for perchlorate
$Qplasma_t$ = AP plasma burned at time t, mg • L $^{-1}$

Equation 1 holds for the heart, thyroid, fat, and egg compartments. The plasma compartment burden is described by the difference equation (2):

$$Qplasma_{t+1} = Qplasma_t - \sum\left(\frac{F \bullet (Qplasma_t / v)}{P}\right) + \sum\left(\frac{F \bullet (Q_t / v)}{P}\right) \quad (2)$$

where,

$Qplasma_t$ = perchlorate plasma burden at time t, mg • L $^{-1}$
$Qplasma_{t+1}$ = perchlorate plasma burden at time $t+1$, mg • L $^{-1}$
Q_t = individual perchlorate compartment burden at time t, mg • L $^{-1}$
F = Blood flow rate into the individual compartments, L/hour
v = Volume of individual compartments, L
P= Individual compartmental partitioning coefficients for perchlorate

The kidney burden may be described as (3):

$$Qkidney_{t+1} = Qkidney_t - \left(\frac{F \bullet (Qkidney_t / v)}{P}\right) - U \bullet Qkidney_t \ldots + \left(\frac{F \bullet (Qplasma_t / v)}{P}\right) \quad (3)$$

where,

$Qkidney_t$ = perchlorate kidney burden at time t, mg • L $^{-1}$
$Qkidney_{t+1}$ = perchlorate kidney burden at time $t+1$, mg • L $^{-1}$
F = Blood flow rate into the kidney, L/hour
v = Volume of kidney, L
P = Compartmental partitioning coefficient for perchlorate,
U = Coefficient for the loss of perchlorate through urinary secretion
$Qplasma_t$ = perchlorate plasma burned at time t, mg • L $^{-1}$

The perchlorate liver burden may be determined by (4):

$$Qliver_{t+1} = Qliver_t - \left(\frac{F \bullet (Qliver_t / v)}{P}\right) - B \bullet Qliver_t \ldots + \left(\frac{FG \bullet (Qgutwall_t / v)}{P}\right) + \left(\frac{F \bullet (Qplasma_t / v)}{P}\right) \quad (4)$$

where,

$Qliver_t$ = perchlorate liver burden at time t, mg • L $^{-1}$
$Qliver_{t+1}$ = perchlorate liver burden at time $t+1$, mg • L $^{-1}$
F = Blood flow rate into the liver, L/hour
v = Volume of liver, L
P = Compartmental partitioning coefficient for perchlorate,
B = Coefficient for the loss of perchlorate through biliary secretion
FG = Blood flow rate for the gut wall, L/hour
$Qplasma_t$ = perchlorate plasma burned at time t, mg • L $^{-1}$
$Qgutwall_t$ = AP gut wall burden at time t, mg • L $^{-1}$

The perchlorate gut wall burden at time t can be described as (5):

$$Qgutwall_{t+1} = Qgutwall_t - \left(\frac{FG \bullet (Qgutwall_t / v)}{P}\right) - D \bullet Qgutwall_t \ldots + \alpha \bullet Qgutcontent_t + \left(\frac{FG \bullet (Qplasma_t / v)}{P}\right) \quad (5)$$

where,

$Qgutwall_t$ = perchlorate gut wall burden at time t, mg • L $^{-1}$
$Qgutwall_{t+1}$ = perchlorate gut wall burden at time $t+1$, mg • L $^{-1}$
F = Blood flow rate of the gut wall, L/hour
v = Volume of gut wall, L
P = Compartmental partitioning coefficient for perchlorate
D = Coefficient for secretion of perchlorate out of the gut wall
FG = Blood flow rate for the gut wall, L/hour
$Qplasma_t$ = perchlorate plasma burned at time t, mg • L $^{-1}$
$Qgucontent_t$ = perchlorate gut content burden at time t, mg • L $^{-1}$
α = absorption coefficient for perchlorate contaminated gut contents

The burden of perchlorate in the gut contents may be determined as follows (6):

$$Qgutcontent_{t+1} = Qgutcontent_t - \beta \bullet Qgutcontent_t - \alpha \bullet Qgutcontent_t \ldots + B \bullet Qliver_t + D \bullet Qgutwall_t + If_t + Iw_t$$

where,

$Qgutcontent_t$ = perchlorate gut content burden at time t, mg • L $^{-1}$
$Qgutcontent_{t+1}$ = perchlorate gut contnet burden at time $t+1$, mg • L $^{-1}$
D = Coefficient for secretion of perchlorate out of the gut wall
B = Coefficient for the loss of perchlorate through biliary secretion
α = Absorption coefficient for perchlorate contaminated gut contents
β = Coefficient for the loss of perchlorate through defecation
If_t = Ingestion rate of perchlorate in food items (mg•h $^{-1}$)
Iw_t = Ingestion of perchlorate in water (mg•h $^{-1}$)

The ingestion rate of perchlorate in food, If_t, (mg•h $^{-1}$) may be written as (7):

$$If_t = \sum_{i=1}^{m} p_i \times Cf_i \times \upsilon_i$$

where

p_i = proportion of total diet contributed by item i at time t
Cf_i = consumption rate of food item i , mg•h $^{-1}$
v_i = perchlorate concentration in food item i , mg•kg $^{-1}$

Similarly, for ingestion of perchlorate in water, Iw_t (mg•h $^{-1}$) is (8):

$$Iw_t = Cw \times \upsilon$$

where

Cw_i = consumption rate of water, L•h $^{-1}$
v_i = perchlorate concentration in food item i , mg•L $^{-1}$

Parameters for the PBTK model were obtained from various sources. Quail organ blood flow rates, weights, and volumes were scaled to represent steady-state quail values (Strukie, 1986). Blood flow rates, weights, and volumes for the egg compartment were also scaled to represent reproductive processes in quail (Freeman, 1984).

The thyroid hormone sub-model was adapted from a model developed for the human thyroid system by DiStefano et al. (1975), DiStefano and Fisher (1976), Saratchandran et al. (1976), and DiStefano and Mori (1977). The number of parameters in the model developed by these authors were reduced or simplified in order to better represent the thyroid hormone system of wildlife species. The model is currently running with

parameter values calibrated to generate output that falls within two percent of the steady-state values as reported by Saratchandran et al. (1976). **Figure 5-177** depicts the flow diagram for the thyroid hormone system sub-model.

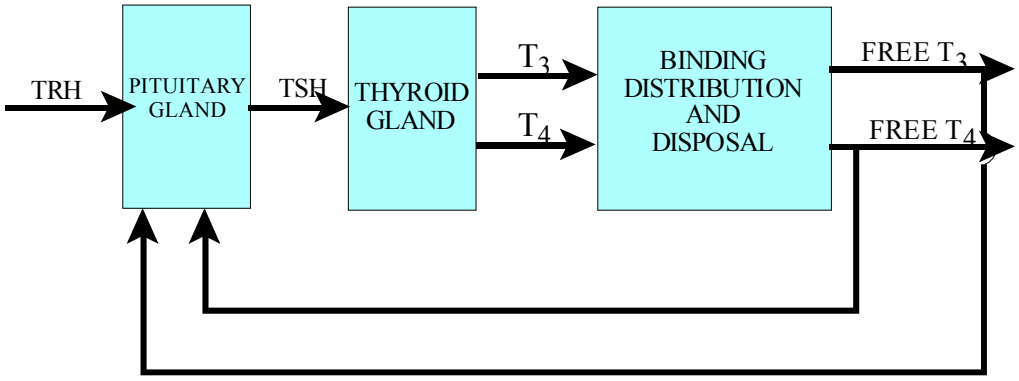

Figure 5-177
Flow Diagram of the Thyroid Hormone System Sub-Model

The modeling objectives were to (1) predict the uptake and distribution of perchlorate in mammal and avian body tissues, (2) predict maternal transfer of perchlorate to eggs, and (3) predict the effects of ammonium perchlorate on the thyroid hormone system (**Figure 5-178**). Internal dose and maternal transfer to egg were related to concentrations of perchlorate in the components of the diets of the avian species feeding in different drainages of the Bosque and Leon watersheds. The model was used to predict the dynamics of perchlorate uptake and distribution, based on stochastic feeding rates, elimination rates, and the effect of perchlorate on thyroid function. Several individuals were simulated to develop a mean distribution of perchlorate in the various tissues at the population level.

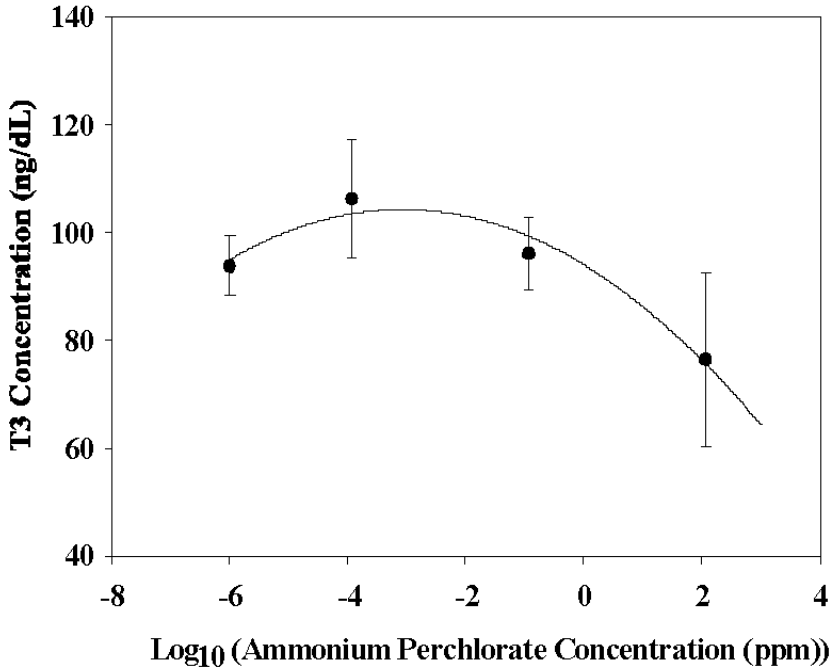

Figure 5-178
Relationship Between T_3 Concentration and Perchlorate Concentration

5.5.1.3.3 Data

5.5.1.3.3.1 Small Mammal Model

Simulations were run for small mammal populations feeding in two drainages of Bosque and Leon River Watersheds representing average and maximum exposures (Station Creek at Highway 107 (T23) and S Creek at Highway 317 (T15), respectively). The highest perchlorate concentrations (range = 67 - 540 ppb) were consistently observed at S Creek at Highway 317 (T15). For comparison purposes, we assumed that small mammals obtained their food and drinking water solely from a single area. Perchlorate concentrations for each area are presented in **Table 5-60**.

5.5.1.3.3.2 Station Creek at Highway 107 (T23)

Simulation output included graphs of perchlorate concentrations in small mammal organs and tissues (**Figure 5-179** and **Figure 5-180**), the secretion rates of T_3 and T_4 (**Figure 5-181**), and the concentration of T_3 and T_4 in plasma and slow and fast pools (**Figure 5-182**).

Table 5-60
Perchlorate Concentrations in Food Items and Drinking Water Used in Model Simulations

	Sampling Areas			
	Station Creek at Highway 107 (T23)		S Creek at Highway 317 (T15)	
	Mean	**S.D.[a]**	**Mean**	**S.D.[a]**
Food (ppm)				
Insects[b]	0.0015	0.0023	1.534	0.725
Plants[c]	30.0	48.24	150.0	26.0
Water (ppb)	24.78	34.20	270.48	157.56

[a] Standard deviation estimate based sample data
[b] LHAAP data
[c] Simulated data using plant model

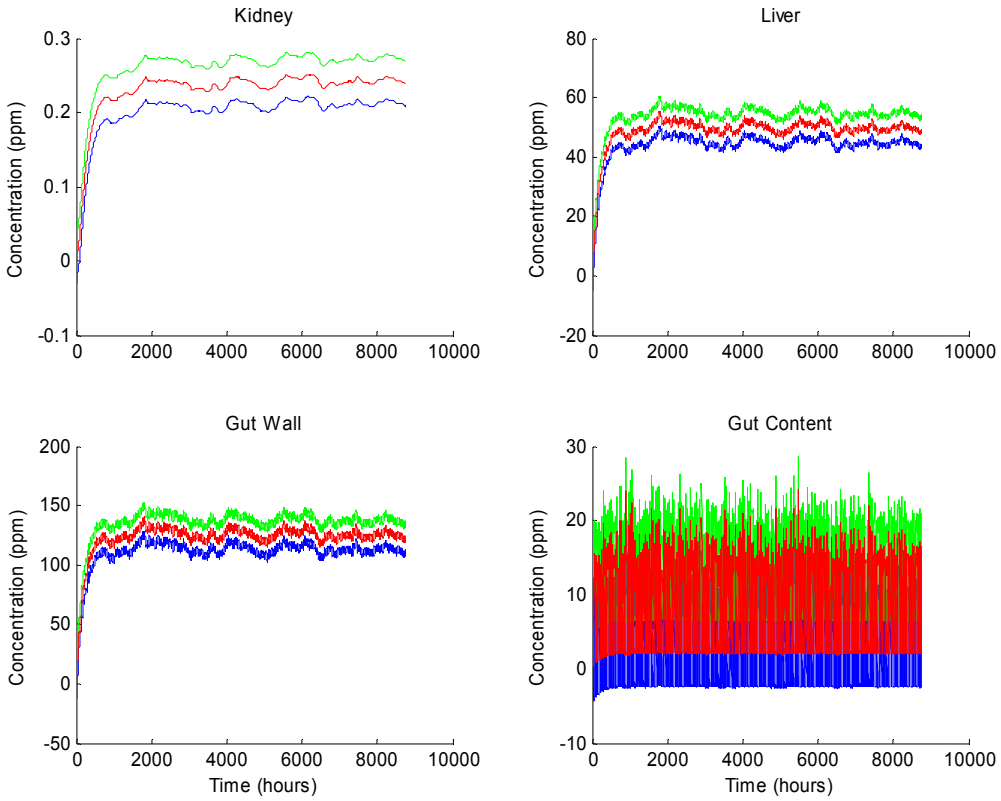

Figure 5-179
Mean Simulated Perchlorate Concentrations (Red) in Small Mammal Kidney, Liver, Gut Wall, and Gut Contents from Station Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

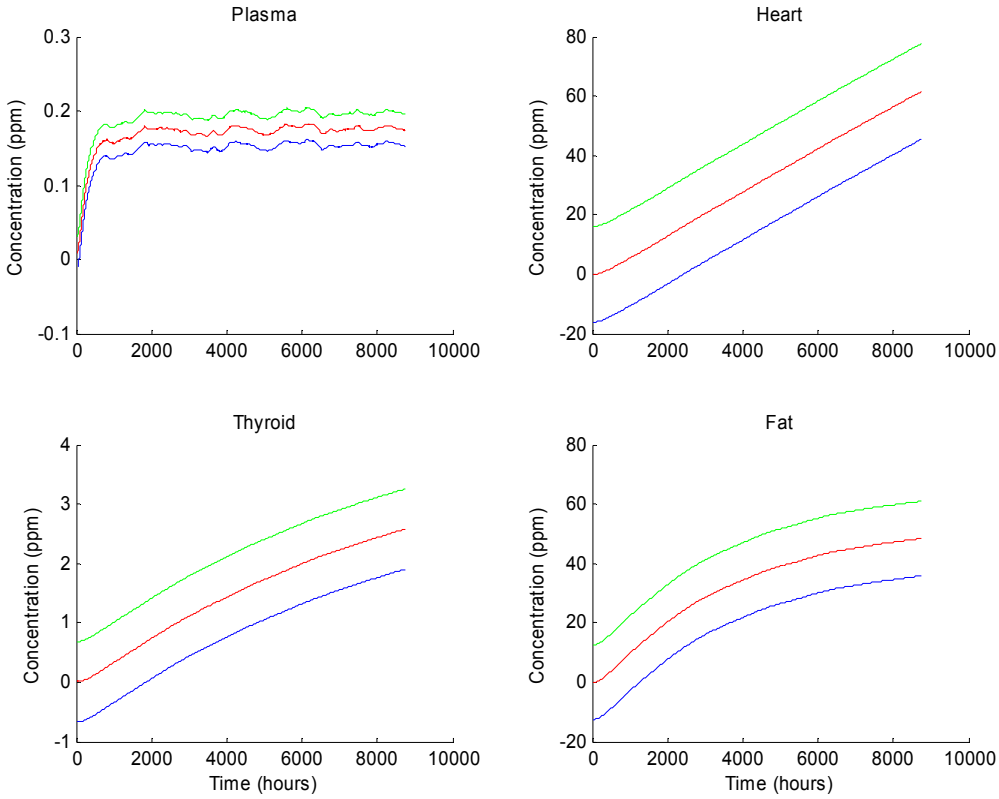

Figure 5-180
Mean Simulated Perchlorate Concentrations (Red) in Plasma, Heart, Thyroid, and Fat Compartments in Small Mammals from S Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

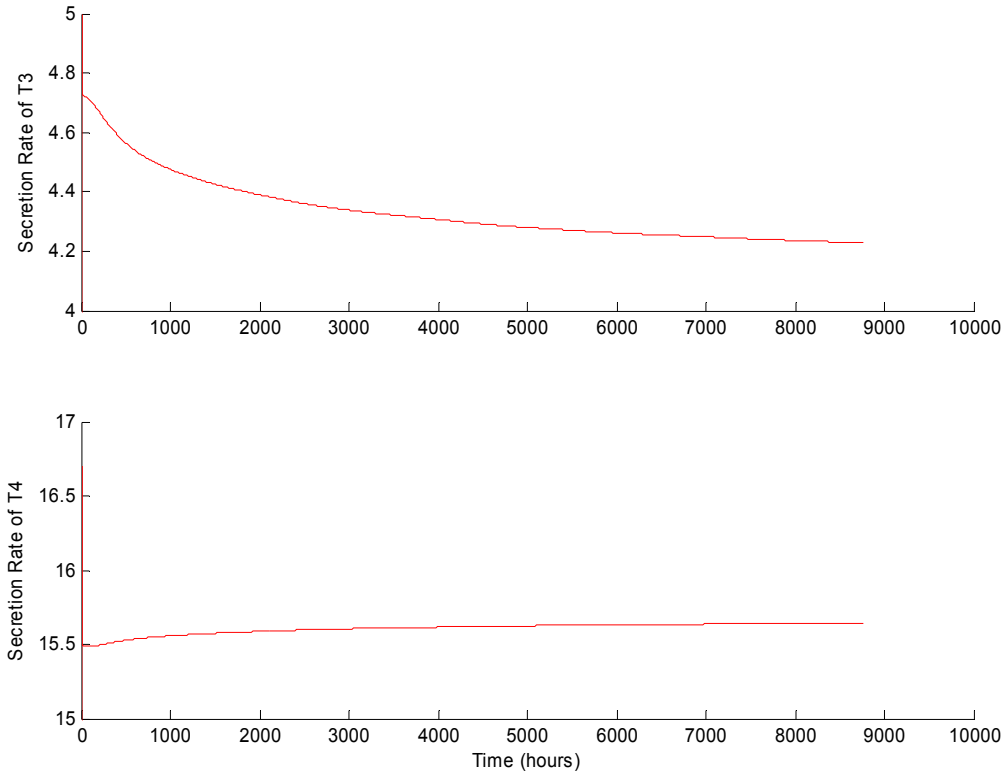

Figure 5-181
Simulated Secretion Rates of T_3 and T_4 Hormones in Small Mammals from Station Creek Drainage

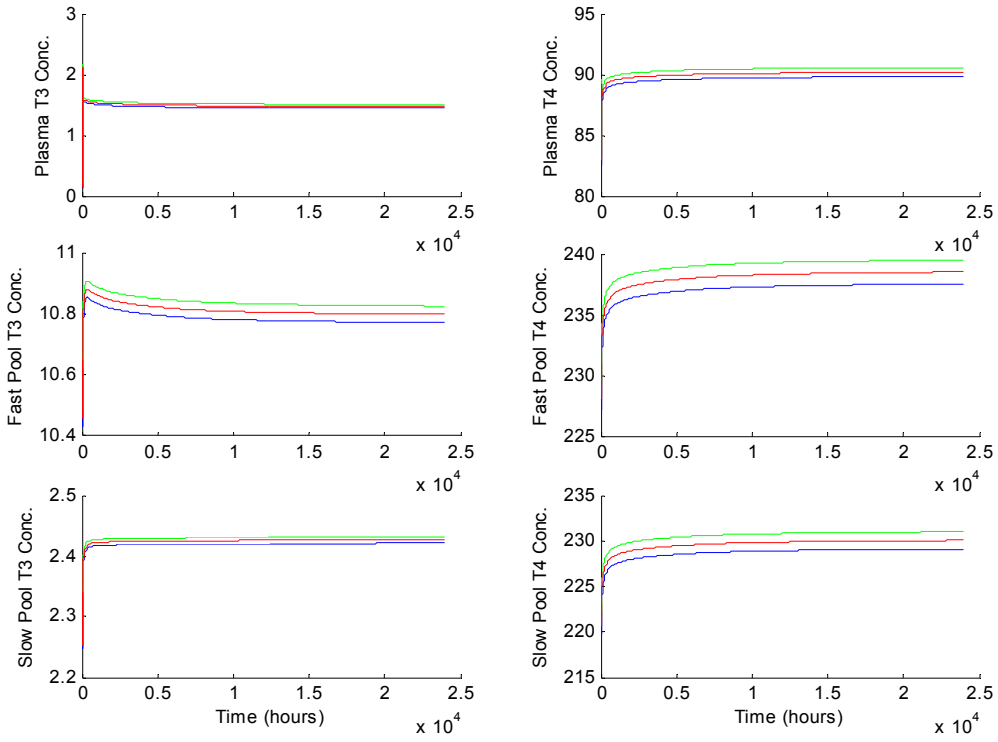

Figure 5-182
Mean Simulated Output of T_3 and T_4 Hormones (Red) in Slow and Fast Pools in Small Mammals in Station Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

5.5.1.3.3.3 S Creek at Highway 317 (T15)

Simulation output included graphs of perchlorate concentrations in small mammal organs and tissues (**Figure 5-183** and **Figure 5-184**), the secretion rates of T_3 and T_4 (**Figure 5-185**), and the concentration of T_3 and T_4 in plasma and slow and fast pools (**Figure 5-186**).

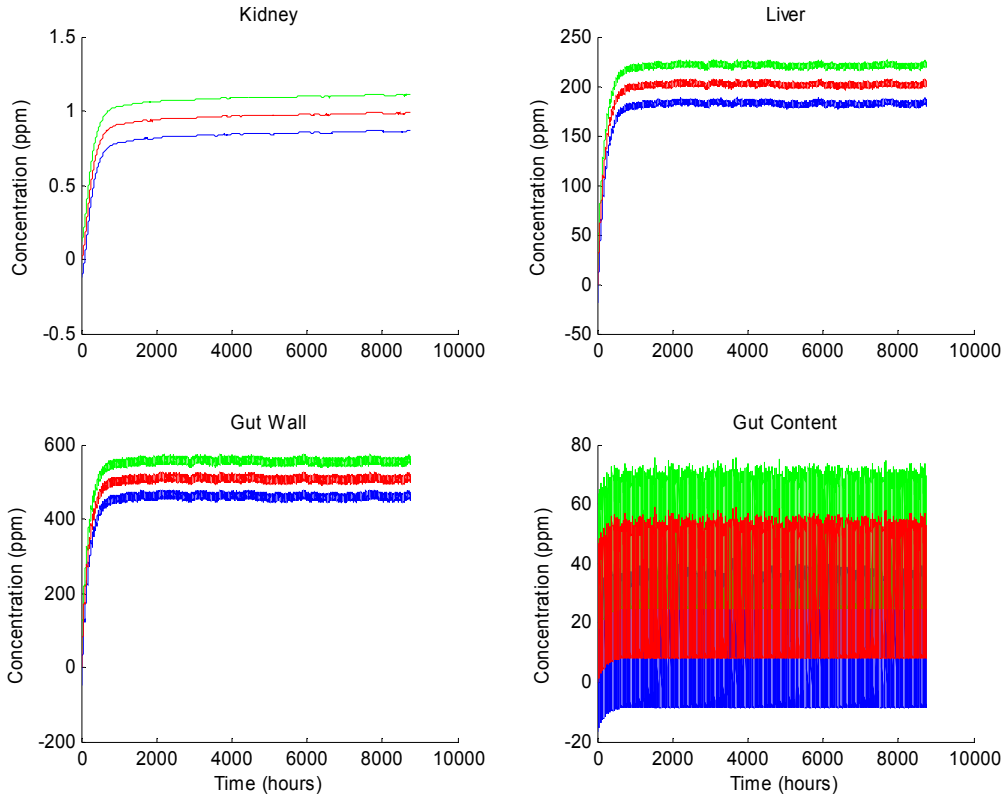

Figure 5-183
Mean Simulated Perchlorate Concentrations (Red) in Small Mammal Kidney, Liver, Gut Wall, and Gut Content Compartments in Small Mammals from S Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

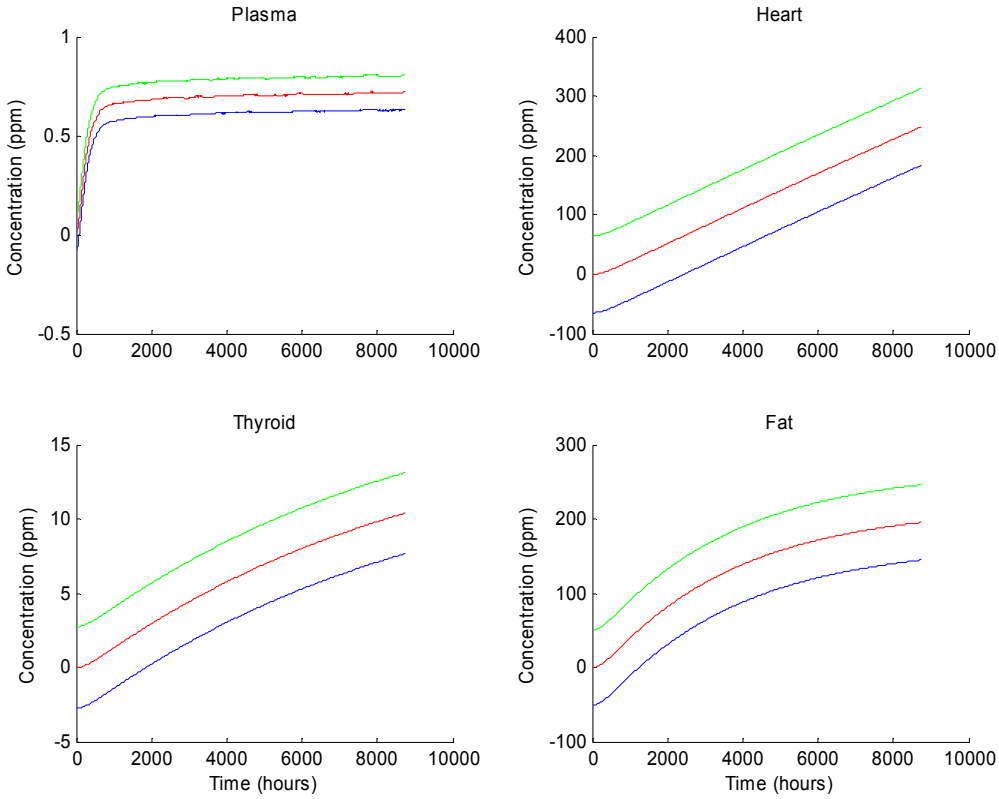

Figure 5-184
Mean Simulated Perchlorate Concentrations (Red) in Plasma, Heart, Thyroid, and Fat Compartments in Small Mammals from S Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

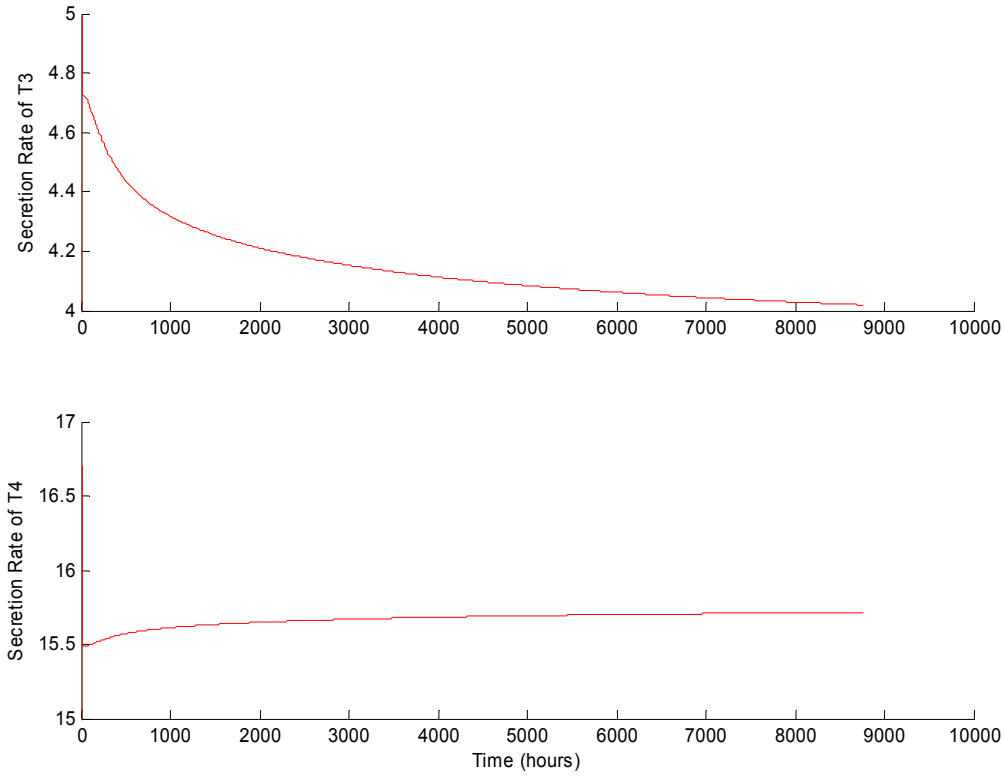

Figure 5-185
Simulated Secretion Rates of T_3 and T_4 Hormones in Small Mammals from S Creek Drainage

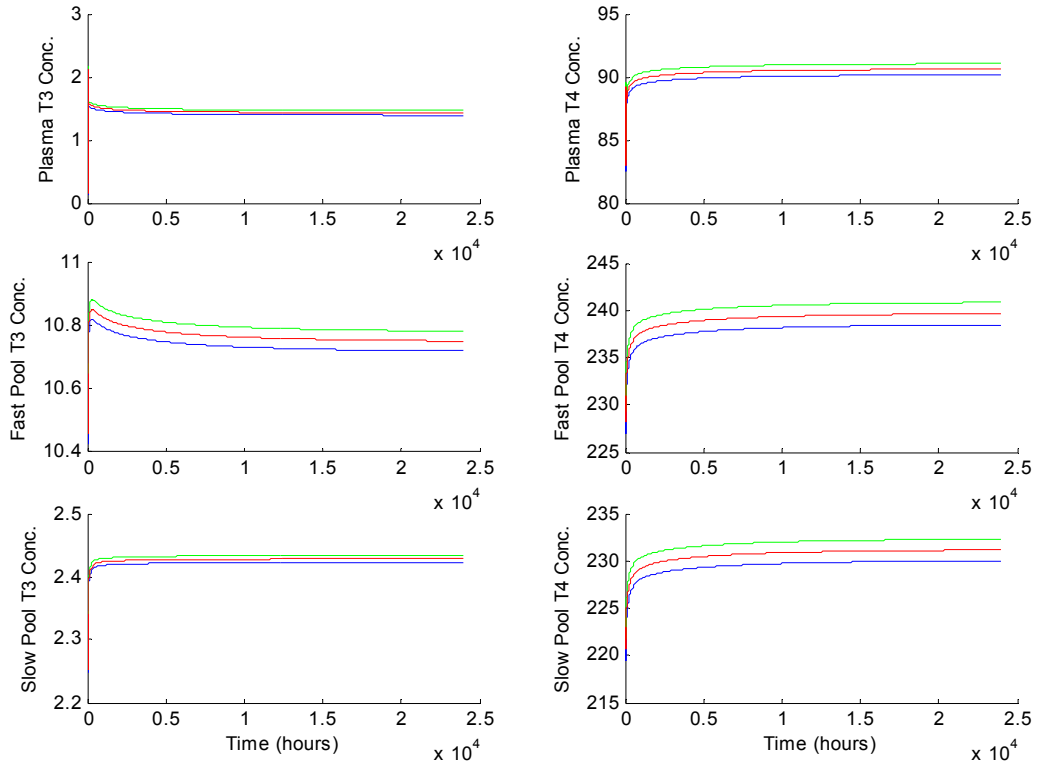

Figure 5-186
Mean Simulated Output of T_3 and T_4 Hormones (Red) in Slow and Fast Pools in Small Mammals in S Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

5.5.1.3.3.4 Bird Model

Simulations were run for quail populations feeding in two drainages of the Bosque and Leon River Watersheds (Station Creek at Highway 107 (T23) and S Creek at Highway 317 (T15)). Each quail population consisted of 100 individuals with a mean body weight of 181g. For comparison purposes, we assumed that the quail obtained their food and drinking water solely from a single area. Internal dose and maternal transfer to egg were related to concentrations of perchlorate in the components of the diets of the avian species feeding in the two different drainages. The model was used to predict the dynamics of perchlorate uptake and distribution, based on stochastic feeding rates, elimination rates, and the effect of perchlorate on thyroid function. The 100 individual birds were simulated to develop a mean distribution of perchlorate in the various tissues. Food item perchlorate concentrations for each area are presented in **Table 5-60**.

5.5.1.3.3.5 Station Creek at Highway 107 (T23)

Simulation output for Station Creek included predicted perchlorate concentrations in quail organs and tissues (**Figure 5-187** and **Figure 5-188**), quail eggs (**Figure 5-189**), the concentrations of TSH in the plasma (**Figure 5-190**), the secretion rates of T_3 and T_4

(**Figure 5-191**), and the concentration of T_3 and T_4 in plasma, and the slow and fast pool compartments (**Figure 5-192**). Examples of slow and fast pool compartments include muscle and liver, respectively. The designations slow and fast pool, were derived from physiological blood flow to various organs. Those organs that were poorly perfused and demonstrated slow blood-flow rates were grouped as slow pool, while those that were richly perfused and demonstrated fast blood-flow rates were grouped as fast pool (Saratchandran et al., 1976). The majority of the simulation output is represented by three different colors. Where applicable, the red trend line represents the mean value of the variable under examination. The green, and blue lines represent the upper-95% and the lower-95% confidence interval for the simulation mean, respectively.

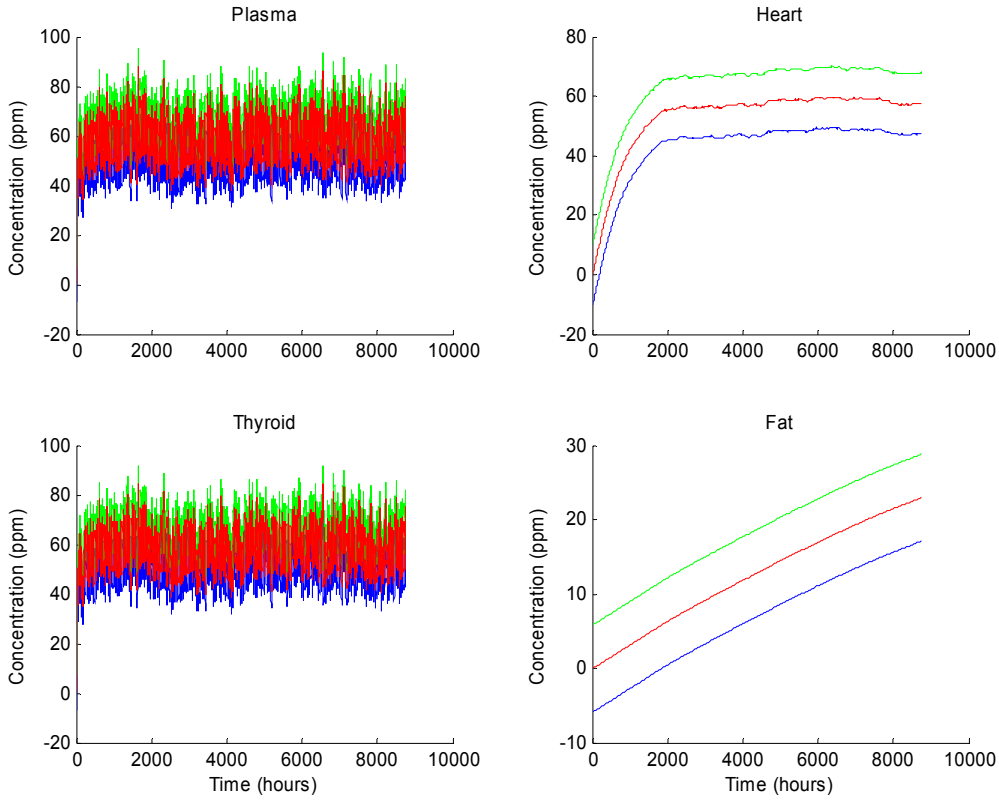

Figure 5-187
Mean Simulated Perchlorate Concentrations (Red) in Quail Plasma, Heart, Thyroid, and Fat from Station Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

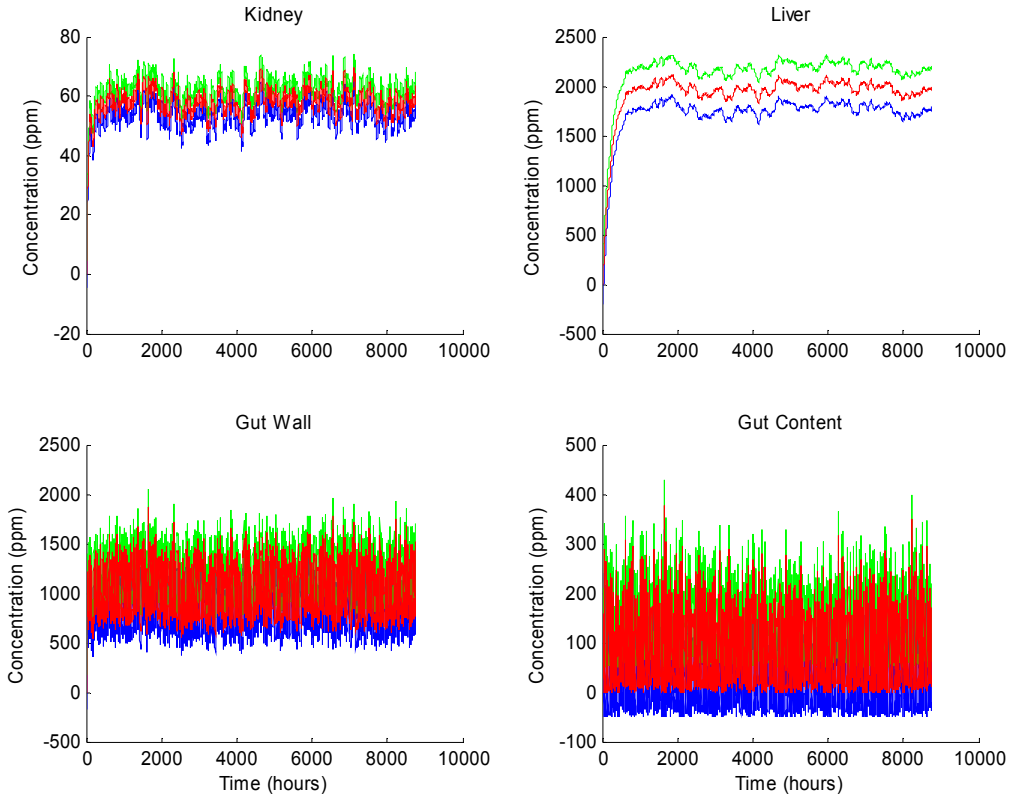

Figure 5-188
Mean Simulated Perchlorate Concentrations (Red) in Quail Kidney, Liver, Gut Wall, and Gut Contents from Station Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

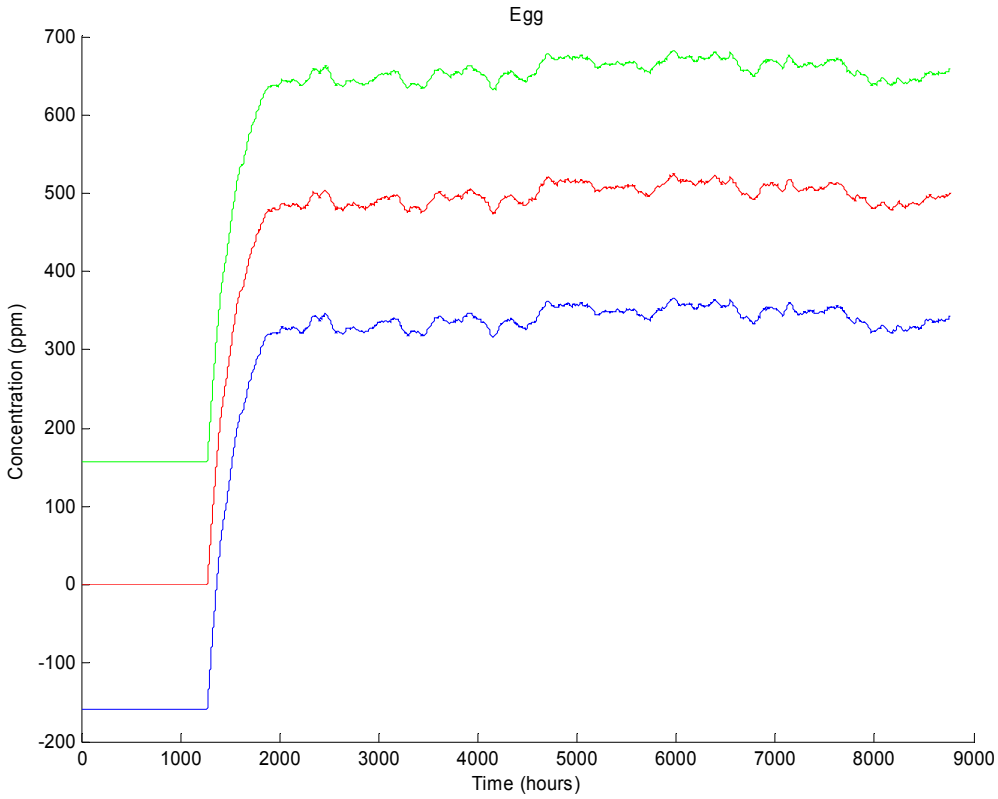

Figure 5-189
Mean Simulated Perchlorate Concentrations (Red) in Quail Eggs from Station Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

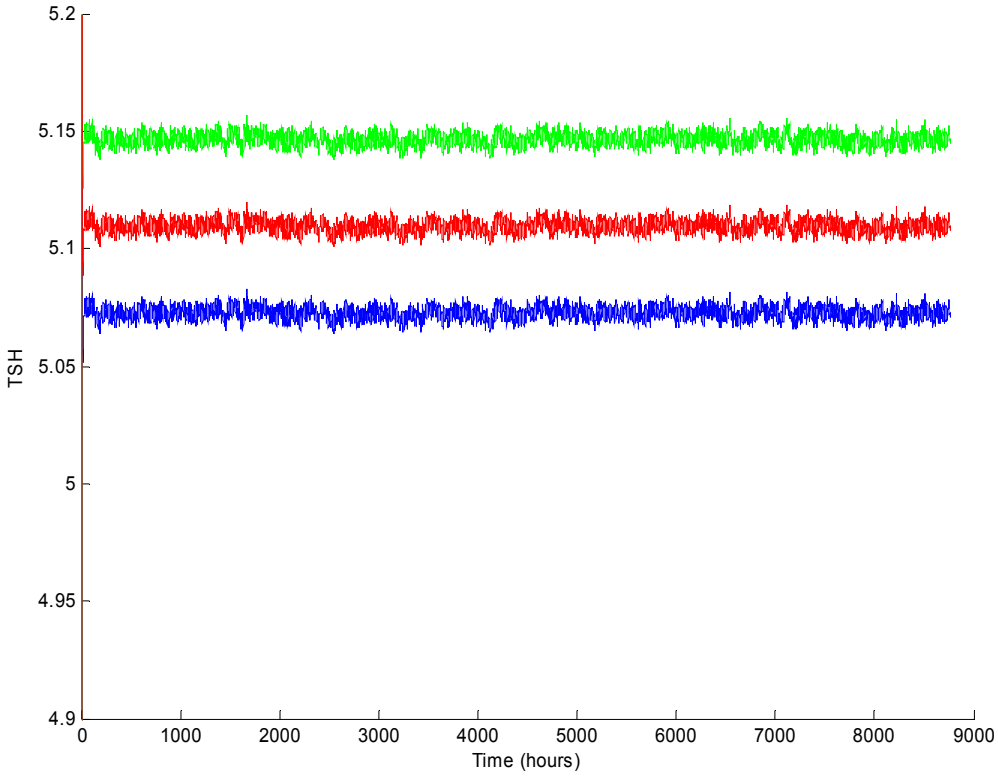

Figure 5-190
Mean Simulated Plasma Concentrations of TSH (Red) for Quail in the Station Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

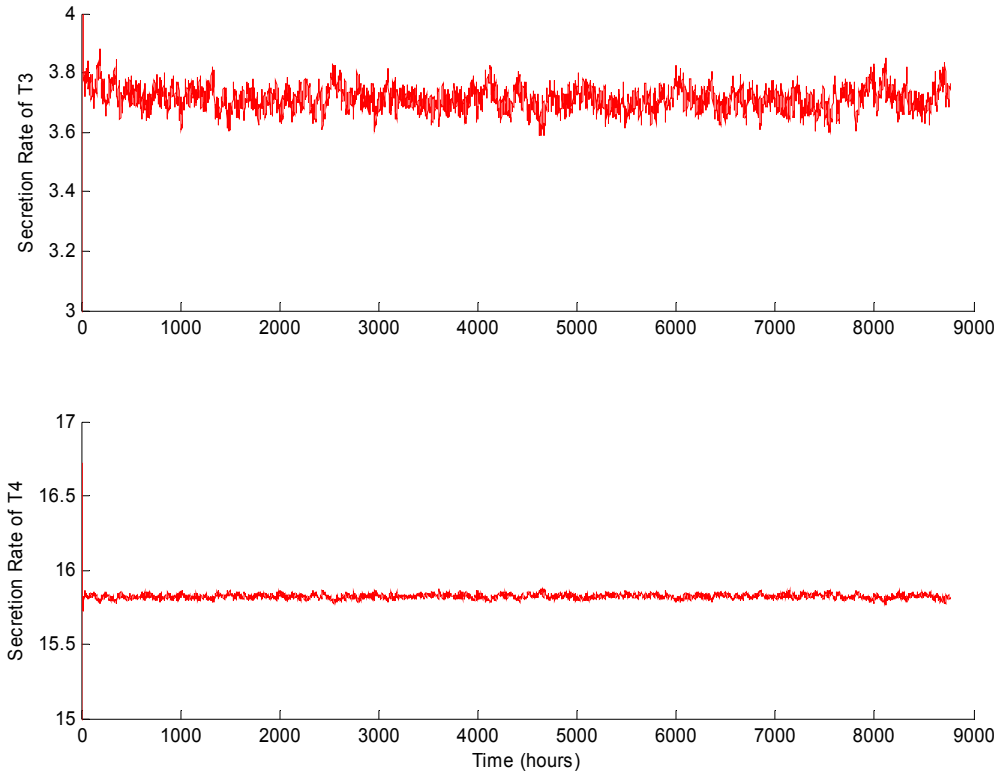

Figure 5-191
Simulated T_3 and T_4 Secretion Rates in Quail from the Station Creek Drainage

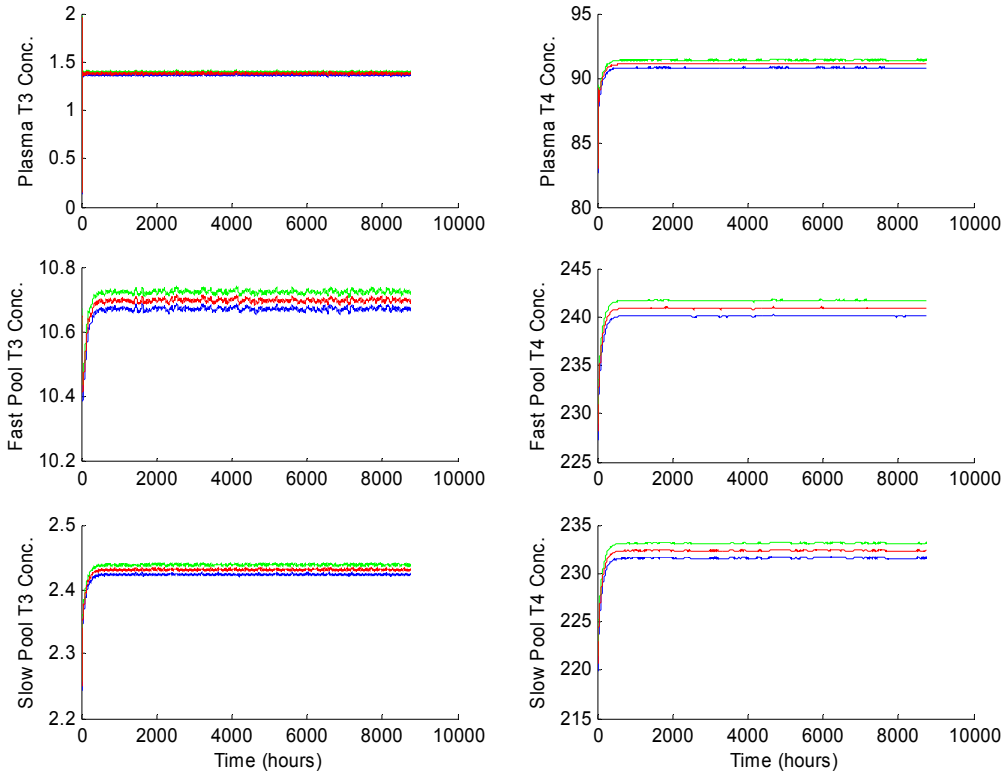

Figure 5-192
Mean Simulated T_3 and T_4 Concentrations (Red) in Quail from the Station Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

5.5.1.3.3.6 S Creek at Highway 317 (T15)

Similarly, simulation output for South Bosque drainage also included predicted perchlorate concentrations in quail organs and tissues (**Figure 5-193** and **Figure 5-194**), quail eggs (**Figure 5-195**), the concentrations of TSH in the plasma (**Figure 5-196**), the secretion rates of T_3 and T_4 (**Figure 5-197**), and the concentration of T_3 and T_4 in plasma and slow and fast pools (**Figure 5-198**).

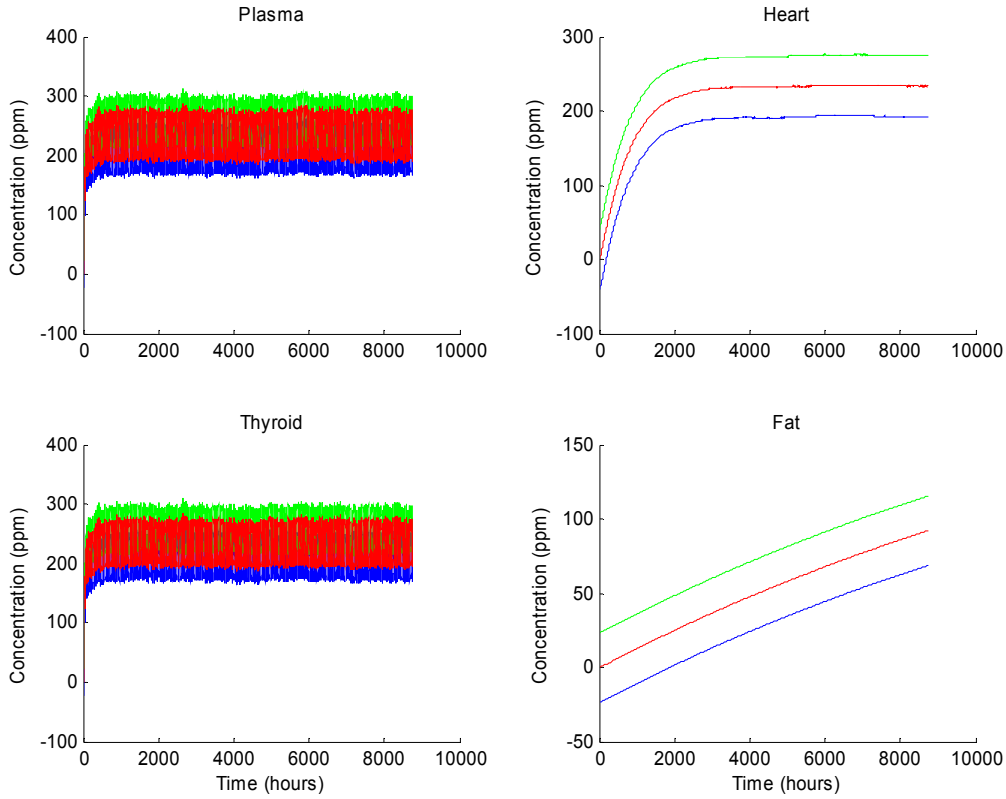

Figure 5-193
Mean Simulated Perchlorate Concentrations (Red) in Quail Plasma, Heart, Thyroid, and Fat from S Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

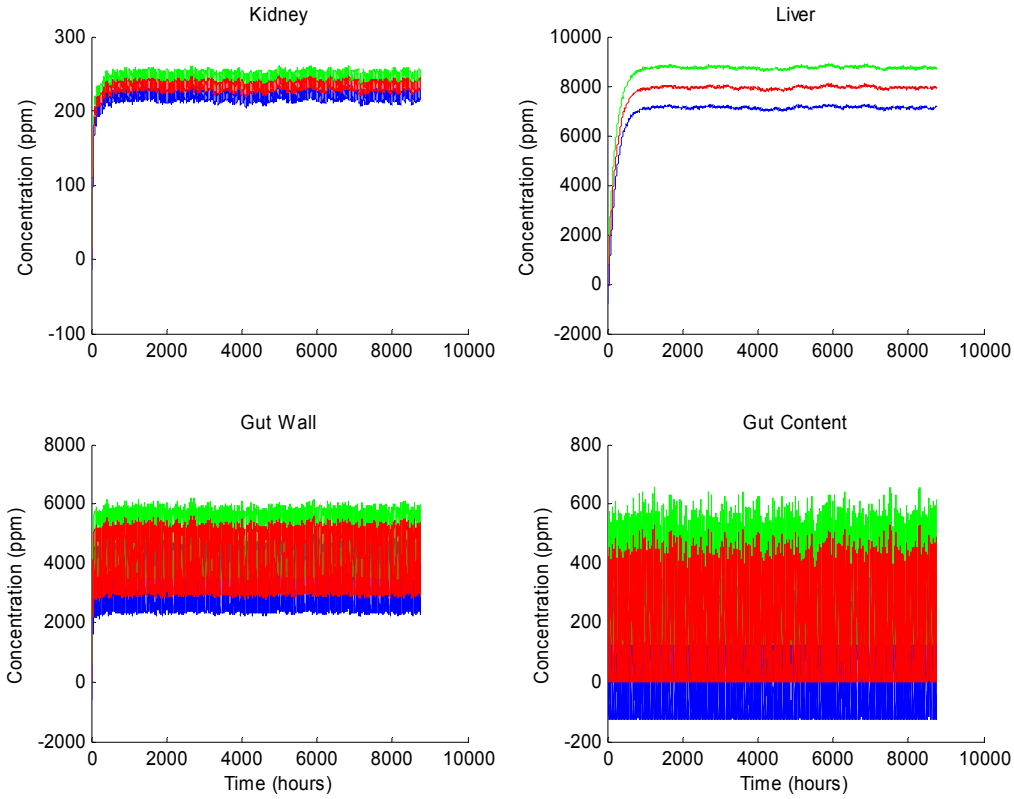

Figure 5-194
Mean Simulated Perchlorate Concentrations (Red) in Quail Kidney, Liver, Gut Wall, and Gut Contents from S Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

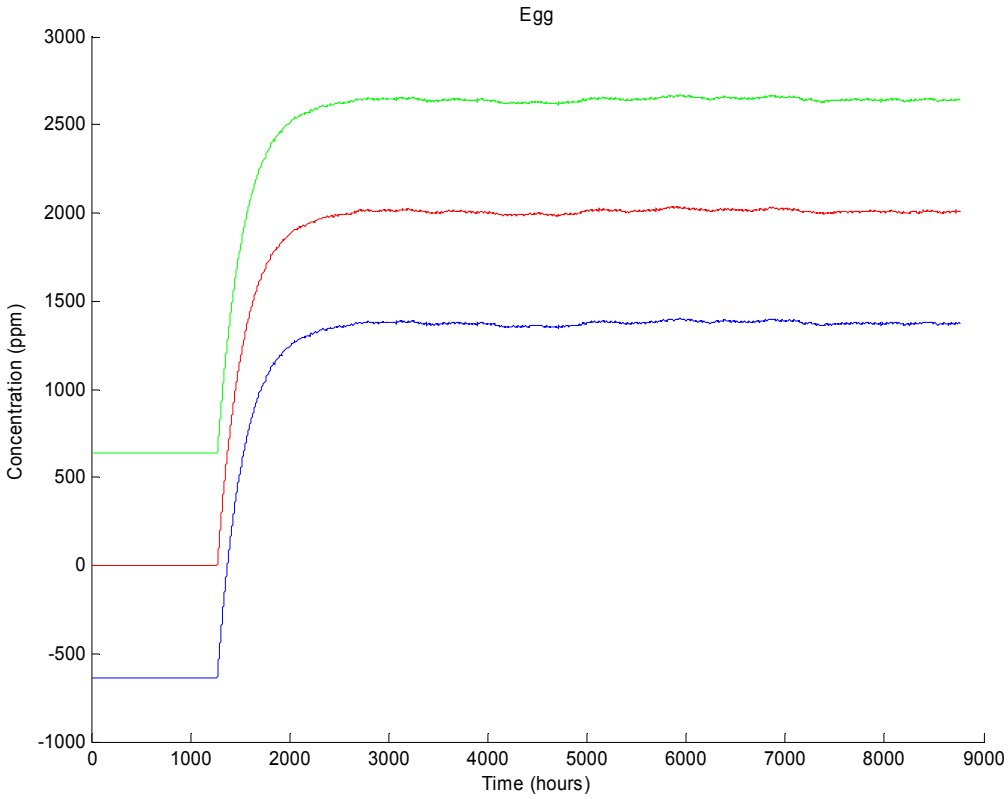

Figure 5-195
Mean Simulated Perchlorate Concentrations (Red) in Quail Eggs from S Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

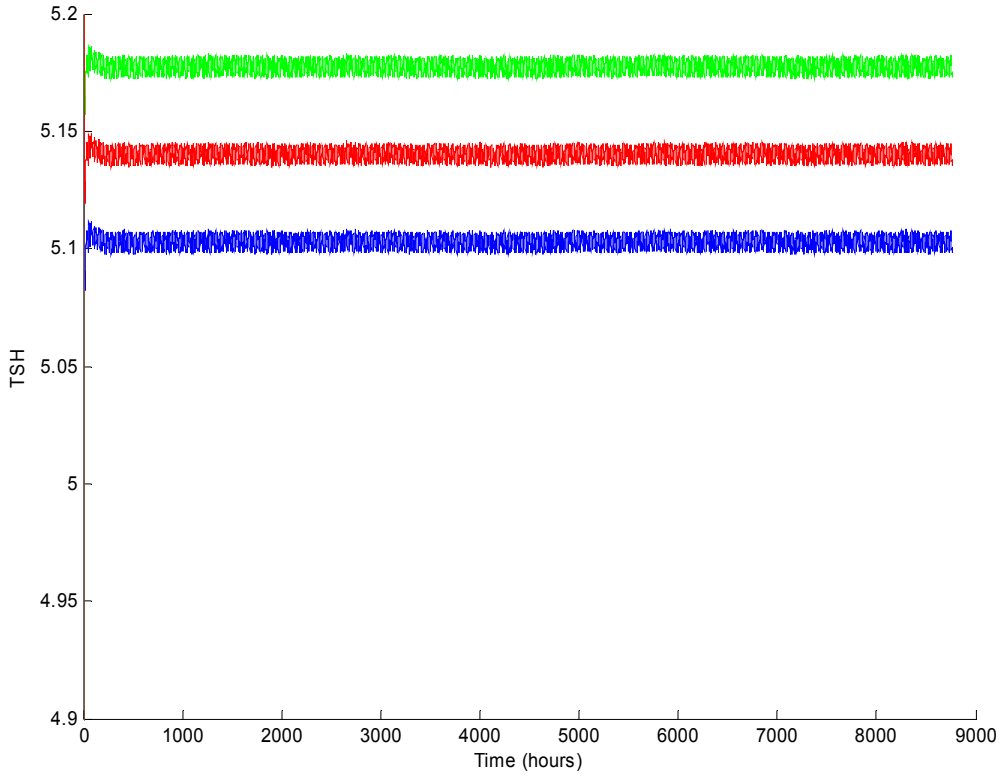

Figure 5-196
Mean Simulated Plasma Concentrations of TSH (Red) for Quail in the S Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

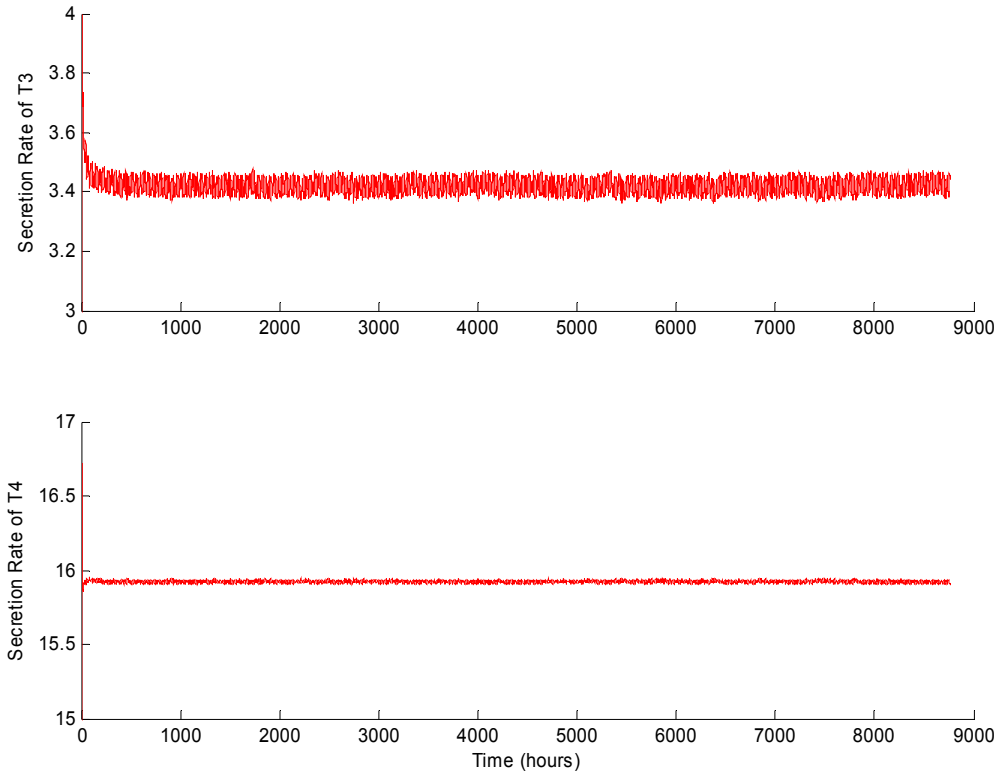

Figure 5-197
Simulated T_3 and T_4 Secretion Rates in Quail from the S Creek Drainage

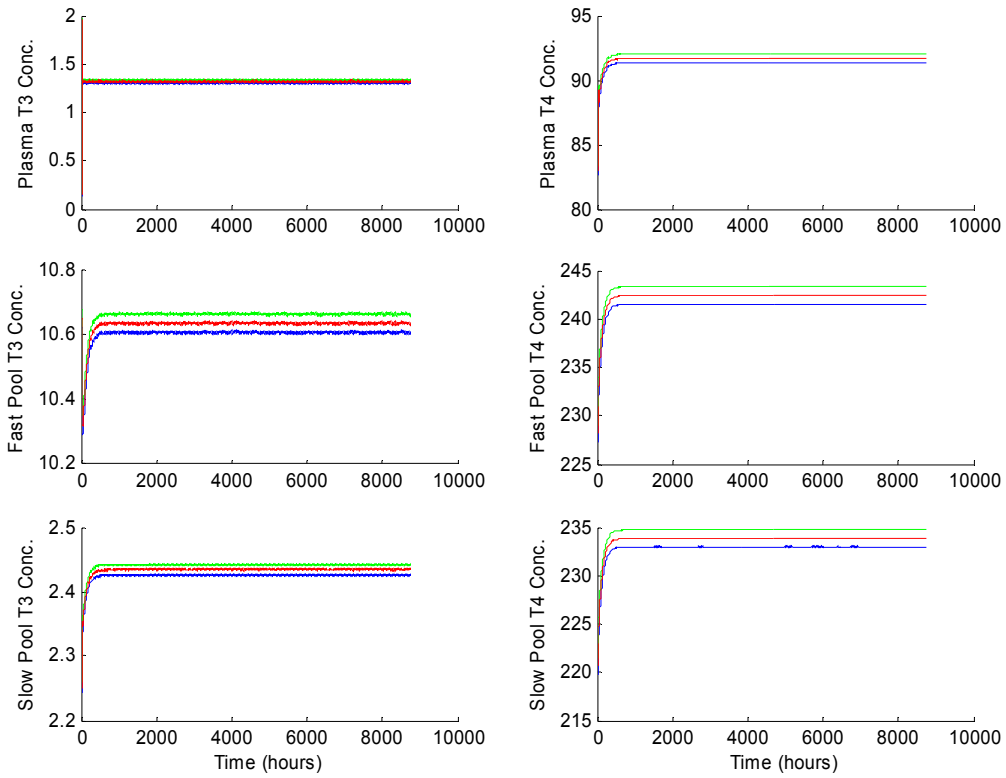

Figure 5-198
Mean Simulated T_3 and T_4 Concentrations (Red) in Quail from S Creek

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

The simulation output is similar in shape for the corresponding variables of both the S Creek and Station Creek Drainages. As the population of birds began to ingest perchlorate-contaminated dietary items, a corresponding perchlorate concentration increase was seen in each compartment. After a given amount of time, the concentration reached equilibrium as the result of a balance between ingested and excreted perchlorate. The process of ingesting and excreting perchlorate also explains the oscillation in each tissue compartment. The up-slope of the oscillation was simply due to ingestion of contaminated food items during the day, while the down-slope is explained by excretion through urine and feces. The model also predicted that higher dietary concentrations of perchlorate resulted in higher tissue concentrations. In addition, it was predicted that perchlorate could be transferred from a female to her eggs.

5.5.1.3.4 Discussion

There were significantly higher perchlorate concentrations predicted in all model small mammal organ and tissue compartments for the South Bosque area, compared with the Station Creek drainage. These differences did not, however, translate to reduced T_3 and T_4 secretion rates or lower T_3 and T_4 concentrations in model compartments. Although parameter estimates are based on literature values, conservative assumptions were made

in their estimation: 1) high transport rates from the liver to the blood, and 2) low elimination rates from the gut. These simulations are only preliminary, but with the current parameter set, additional simulation experiments showed reduced thyroid activity only with order-of-magnitude greater perchlorate concentrations than those in **Table 5-60**.

The model predicts significantly higher perchlorate concentrations in all modeled organ and tissue compartments for the S Creek drainage area compared with the Station Creek drainage area. In attempting to determine if there was a dose-dependent response in thyroid function as it relates to perchlorate concentrations in the thyroid gland, the simulations produced varying results. The plasma TSH level was only marginally reduced, while the various T_3 and T_4 concentrations displayed divergent results. The T_3 secretion rate increased significantly in birds residing in both drainages as compared to the baseline values. Subsequently, the plasma and fast pool T_3 concentrations also increased for both populations, while no significant change became evident for the slow pool T_3 concentration. The resultant T_4 secretion rate decreased for both populations when compared to the baseline values. Additionally, the plasma, fast pool, and slow pool concentrations of T_4 declined as well. Throughout the simulation process, it became apparent that fluctuating perchlorate concentrations at the thyroid did result in fluctuating TSH blood concentrations, T_3 and T_4 secretion rates, as well as T_3 and T_4 concentrations in model compartments. Although the model's output is not necessarily representative of actual hormone levels, the model does suggest that there is a dose-dependent response in thyroid function that is related to perchlorate concentrations at the thyroid gland. It is important to reiterate that the thyroid hormone submodel was based on mammalian studies and the assumption was that the avian thyroid system functioned similarly. Due to the high level of uncertainty in many of the parameters, it is important that these results not be interpreted as definitive. By incorporating more laboratory data into the calibration process, as the data become available, the predictive power of the model could be enhanced.

The predicted concentrations as a result of maternal transfer to eggs are within the same range as the predicted concentrations in the adult thyroid compartment. While the actual effects are unknown at this time, it is reasonable to assume that there is risk for altered thyroid function in the developing embryo resulting from the presence of perchlorate in the egg. This altered thyroid function could result in decreased egg viability or possible teratogenic effects. The egg compartment provides output closer to expected real values, but caution must be utilized here as well. Monitoring data from other sites indicate that perchlorate is rarely detected in eggs.

5.5.2 Medium Mammals

Perchlorate can be detected in soils at many contaminated sites, but it is most often associated with ground and surface water bodies. Therefore aquatic organisms including fish and amphibians are considered to be the most likely ecological receptors. However, perchlorate has been detected in terrestrial organisms as well (Smith et al., 2001), indicating that exposure pathways to terrestrial organisms exist. Since raccoons (*Procyon lotor*) consume many of these organisms and forage along water bodies, they were

chosen as a potential biomonitoring species to assess perchlorate exposure among medium-sized mammals in the Bosque/Leon River watershed areas that have been impacted by the NWIRP facility. Raccoons are closely associated with the terrestrial/aquatic interface and are common throughout much of Texas. Perchlorate exposure among raccoons may result from consumption of contaminated water or food items. Raccoons consume approximately 0.083 g water/ g body weight/ day along with a diet principally composed of plant and animal matter (insects, amphibians, crayfish, fish, rodents; USEPA, 1993). Previous research conducted at another contaminated site (Longhorn Army Ammunition Plant, Karnack, Texas) indicated that perchlorate can be detected in several of these food sources at various levels (Smith et al., 2001). Diets of raccoons may also contain a significant amount of soil (as much as 9.4%) from feeding and grooming (Beyer et al., 1994). Opossums (*Didelphis virginianus*) are a non-target species that is often captured when trapping raccoons. Since they inhabit much of the same habitat and consume many similar types of food items, they too may be suitable indicators of perchlorate contamination and subsequent exposure.

Based on the potential for exposure through water, food item, and soil ingestion, raccoons were selected as a biomonitoring species for this study. We hypothesized that raccoons (and opossums) would be exposed to perchlorate through consumption of food, water, and soil along impacted water bodies in the Bosque/Leon River watershed study area and that thyroid hormone concentrations would be reflect perchlorate exposure.

5.5.2.1 Perchlorate Residue in Native Medium Mammals

5.5.2.1.1 Methodology

Raccoons and opossums were trapped from January 9-10, 2003 using live-traps. Traps were set in areas with some degree of perchlorate contamination as determined by previous water and vegetation monitoring. Areas trapped included Station Creek (T23), the unnamed tributary near the wastewater treatment plant at Highway 317 (T14), the South Bosque at Highway 317 (T16), S Creek at Highway 317 (T15), and Harris Creek (T19). These areas are shown on **Figure 5-199**. Traps were baited with sardines, covered with vegetation, and placed at locations frequented by raccoons (as noted by tracks). Traps were set in the evening (ca. 1700 hrs) and checked the following morning (ca. 0600-0900 hrs).

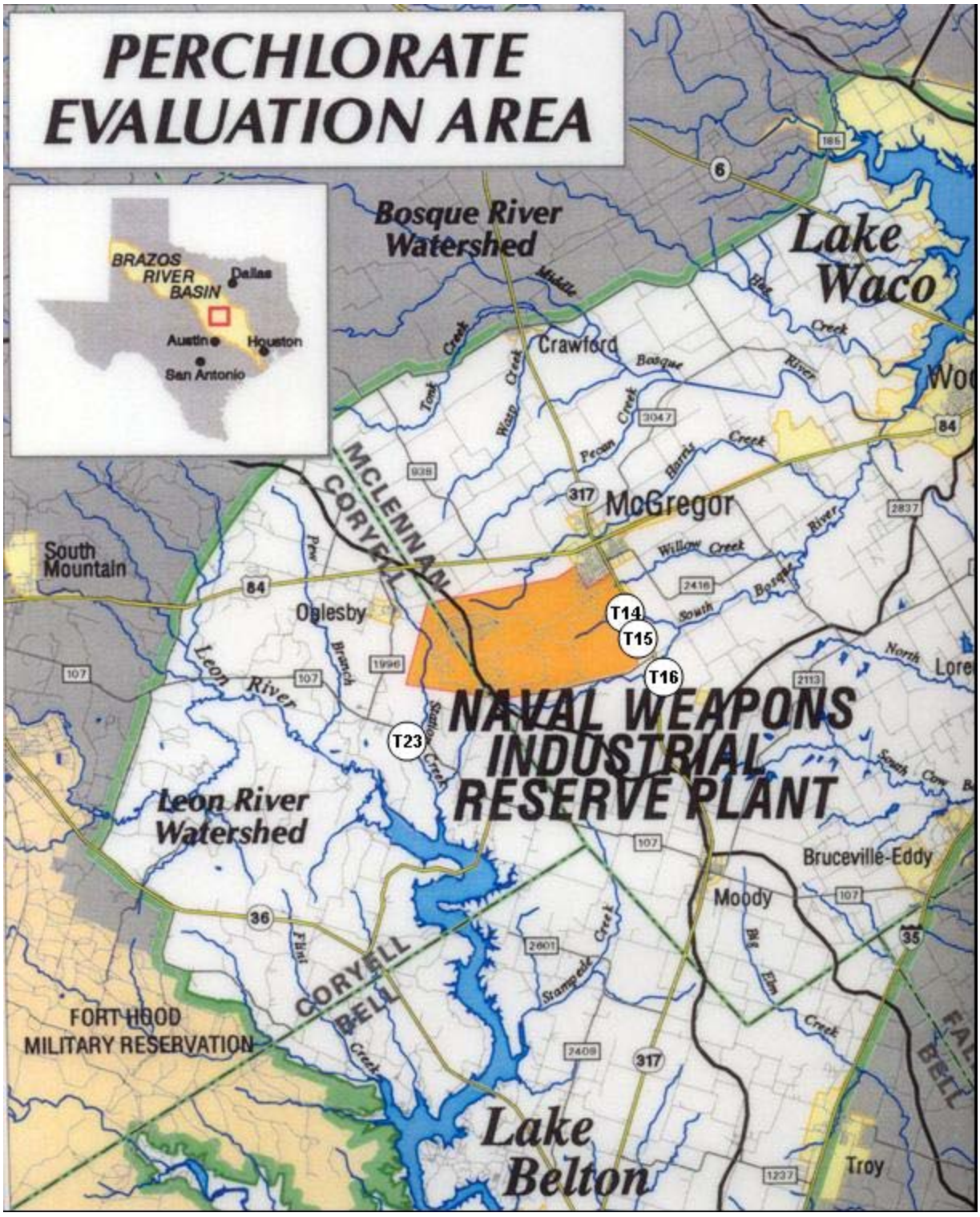

Figure 5-199
Map of Study Area Illustrating the Approximate Locations where Medium Mammal Samples Were Collected

Upon capture, raccoons and opossums were transferred to a squeeze cage and weighed using a Pesola scale calibrated to the nearest 0.5 kg. Weight of the animal was recorded and used to calculate the appropriate dose of ketamine hydrochloride (8-10 mg/kg) and xylazine hydrochloride (2 mg/kg) needed to sedate the animal. The sedative was injected intramuscularly into the left or right hip while animals were constrained in squeeze cages. Ophthalmic ointment was applied to the animal's eyes to keep them moist. Approximately 3 to 8 mL of blood, depending on body size, was taken from the jugular vein, placed in plasma tubes (containing EDTA), and centrifuged for 12 minutes. Plasma samples were frozen (-80 °C) until hormone and residue analysis could be performed. Animals were euthanized via overdose of sodium pentathol and necropsied. Thyroids were removed, placed in a triple aldehyde electron microscopy fixative (Tandler, 1990) for 10 minutes and stored in formalin until histopathology could be performed.

Plasma was analyzed to determine the presence of perchlorate using ion chromatography (EPA Method 314.0; Hautman et al., 1999) with slight modifications. Briefly, analytical samples were prepared for analysis by combining 5 mL ethanol and 1 mL raccoon plasma in a glass tube, mixing the solution for 5 seconds on a vortex and centrifuging for 10 minutes to isolate the supernatant. Then supernatants were decanted into new test tubes and vortex-evaporated until the level of supernatant was approximately 0.5 mL. The supernatant was then brought to a volume of 5 ml with Milli-Q water and filtered (0.45 μm). The final sample was then analyzed for perchlorate using ion chromatography (Anderson and Wu, 2002) (**Appendix X**).

5.5.2.1.2 Data

Medium mammal sampling efforts focused on areas where perchlorate had been detected in surface water and vegetation. The results of residue analyses of raccoon and opossum blood plasma indicate that despite the presence of perchlorate in drinking water at these sites, there was no quantifiable exposure measured in blood (**Table 5-61**).

Table 5-61
Perchlorate Residues in Plasma Samples from Raccoons and Opossum Collected from Areas near NWIRP

Species	Capture Location: UTM: ID[a]	Perchlorate (ng/mL)
Raccoon	Unnamed tributary near wastewater treatment plant at Highway 317: 652853E 3476589N: T14	ND
	S Creek at Highway 317: 653646E 3474993N: T15	ND
	Station Creek at Highway 107: 642988E 3471304N: T23	ND
	Unnamed tributary near wastewater treatment plant at Highway 317: 652853E 3476589N: T14	ND
	S Creek at Highway 317: 653646E 3474993N: T15	ND
Opossum	Station Creek at Highway 107: 642988E 3471304N: T23	ND
	South Bosque at Highway 317: 653810E 3473908N: T16	ND
	S Creek at Highway 317: 653646E 3474993N: T15	ND
	S Creek at Highway 317: 653646E 3474993N: T15	ND

[a]see **Figure 5-199** for approximate capture locations.

5.5.2.1.3 Discussion

Despite the likely occurrence of perchlorate exposure in raccoons and opossums, perchlorate residues in blood plasma (if present) were below the limit of detection. As the surface to volume ratio of an animal decreases, its relative exposure decreases. In other words, larger animals do not receive as large of an exposure (on a surface to volume ratio basis) as small animals. This toxicological concept likely contributed to the results observed for medium mammals. Additionally, perchlorate is excreted rapidly after exposure, and thus may have been eliminated from captured animals prior to collection of blood samples.

5.5.2.2 Thyroid Hormones and Histology in Native Medium Mammals

5.5.2.2.1 Hormone Methodology

Plasma samples were analyzed for T_3 and T_4, subject to the availability of plasma from individual animals. Coat - A- Count Total kits (TKT31 and TKT41; Diagnostic Products, Los Angeles, CA, USA) were used to measure T_3 and T_4 levels. In order to conserve plasma, all assay optimization (Newell et al., 1982) was completed using raccoon serum collected during a previous study.

The basic protocol of the T_3 kit was followed with slight modifications. The volume of plasma was optimized by increasing the amount used in each kit to achieve 40% binding (Chard, 1990). The optimal and most efficient use of plasma was determined to be 150 µL for T_3. Calibration points for the T_3 standard curve were 0, 20, 50, 100, 100, 200, and 600 ng/mL. Optimization of the T_4 kit was conducted in the same manner, and the optimal plasma volume was determined to be 100 µL. Calibration points for the T_4 standard curve were 0, 1, 4, 10, 16, and 24 µg/dL. Since raccoon plasma volumes used in each assay were greater than those specified by the kits, actual hormone concentrations would be lower than the values reported herein. Therefore, relative values are reported and should not be construed as actual raccoon thyroid hormone concentrations.

5.5.2.2.2 Histology Methodology

A Tissue-Tek V.I.P. 2000 Processor was used to process the thyroid tissues, which were then embedded in paraffin (Miles Scientific). Blocks containing the thyroid tissues were then cut into sections at 7 µm with a microtome (Cut 4055, Olympus America Inc.). Tissue sections were then mounted on glass microscope slides and stained following basic hematoxylin and EosinY staining technique (Ross, 1995).

Slides were examined with an Olympus BX52 compound light microscope with attached Olympus DP1-L digital camera and control pad (Olympus Optical Co., Ltd). Photographs of each specimen were taken at 40X magnification. Sections were selected from the slides containing thyroid tissue from each raccoon. Thyroid tissue was examined and scored for the amount of hyperplasia, hypertrophy and colloid depletion present based upon the guidelines established by the Pathology Working Group (Mann, 2000). Each trait was given a score of 0 for no indication of thyroid damage, 1 for slight damage, or 2 for severely damaged (Mann, 2000). An average score for all three traits was then

determined for individual voles and each of the three treatment groups by generation and sex. Additionally, the number of microfollicles present was recorded.

5.5.2.2.3 Data

Five raccoons and four opossums were collected from four areas around the NWIRP that had previously produced water or other environmental samples containing perchlorate in January, 2002. All raccoons and opossums appeared to be in good general condition, and no abnormalities were noted. Upon inspection, all thyroid glands appeared to be of normal color, size, and shape.

Raccoon plasma triiodothyronine (T_3) concentrations ranged from 49.85 ng/dL to 101.97 ng/dL, and thyroxine (T_4) concentrations ranged from 4.25 µg/dL to 7.20 µg/dL (**Table 5-62**). Mean raccoon T_3 and T_4 concentrations were 73.9 ± 8.57 ng/dL and 6.3 ± 0.56 µg/dL. Thyroid hormone concentrations among raccoons inhabiting the perchlorate study near the NWIRP site are higher than the mean thyroid hormone concentrations in raccoons (56.53 ± 3.1 ng/dL and 3.57 ± 0.25 µg/dL, respectively) inhabiting the Longhorn Army Ammunition Plant (LHAAP) in Karnack, Texas. Mean T_3 and T_4 concentrations for opossums were 45.13 ± 4.70 ng/dL and 1.73 ± 0.22 µg/dL, respectively.

Table 5-62
Raccoon and Opossum Triiodothyronine (T_3) and Thyroxine (T_4) Concentrations

Species	Capture Location: UTM: ID[a]	T3 (ng/dL)	T4 (µg/dL)
Raccoon	Unnamed tributary near wastewater treatment plant at Highway 317: 652853E 3476589N: T14	75.17	7.20
	S Creek at Highway 317: 653646E 3474993N: T15	64.75	4.25
	Station Creek at Highway 107: 642988E 3471304N: T23	49.85	6.13
	Unnamed tributary near wastewater treatment plant at Highway 317: 652853E 3476589N: T14	78.10	7.20
	S Creek at Highway 317: 653646E 3474993N: T15	101.97	6.90
Opossum	Station Creek at Highway 107: 642988E 3471304N: T23	39.54	1.22
	South Bosque at Highway 317: 653810E 3473908N: T16	55.18	1.71
	S Creek at Highway 317: 653646E 3474993N: T15	50.73	1.69
	S Creek at Highway 317: 653646E 3474993N: T15	35.05	2.30

[a]see **Figure 5-199** for approximate capture locations.

In addition to thyroid hormone analysis, thyroid glands from all raccoons and opossums were examined histologically. Thyroid gland sections were examined for the presence of hyperplasia, hypertrophy and colloid depletion. No histological abnormalities were detected in any of the raccoon or opossum tissues.

5.5.2.2.4 Discussion

These data suggest that medium-sized mammals inhabiting areas adjacent to the NWIRP and affected streams are not exposed to perchlorate at concentrations high enough to result in physiological effects. This study examined medium-sized mammals during the winter. Food item selection and water consumption differ seasonally among these organisms (USEPA, 1993), therefore alternative exposure rates may occur at other times of the year. In the spring and summer, raccoons and opossums rely more heavily on vegetation and fruits. Given the potential for perchlorate uptake into vegetation, these animals may be exposed to higher concentrations of perchlorate in spring and summer. However, medium-sized mammals do not appear to be at risk outside the NWIRP facility due to their extensive foraging ranges and limited spatial distribution of perchlorate contamination.

5.5.3 Large Mammals

Cattle inhabiting pastures near perchlorate-contaminated water bodies may be at risk for exposure through drinking water or consumption of plant matter grown in the presence of perchlorate. Cattle are obligate herbivores, and as such may be at risk for perchlorate exposure and subsequent effects since perchlorate can accumulate to high concentrations in plants. Wild birds and rodents collected from contaminated terrestrial environs have been found to contain elevated concentrations of perchlorate in tissues (Smith et al., 2001).

Beef and dairy cattle may represent a direct exposure pathway to humans. Therefore, we evaluated perchlorate exposure among cattle inhabiting a pasture in the study area that had a constant influx of perchlorate. We examined exposure via plasma and tissue (consumable beef samples) concentrations and thyroid hormone responses. The objective of this study was to assess potential human exposure to perchlorate through beef cattle inhabiting the Bosque/Leon River watershed study area.

5.5.3.1 Perchlorate Residue in Large Mammals

5.5.3.1.1 Methodology

5.5.3.1.1.1 Study Description and Sample Collection

The study was conducted over 14 weeks during the spring of 2003. Heifer calves (~ 535 lbs. each) were purchased commercially and held for 1 week prior to being placed on two sites (2 heifers per site) near McGregor, McLennan County, Texas. The reference site was a pasture a sufficient distance from the facility such that perchlorate was not expected to be present in surface or groundwater. The treatment site was a pasture near the facility boundary with a spring-fed stream bisecting the property (between locations T18 and T19). Previous monitoring data for the spring indicated that perchlorate was present (20-60 ng/mL). Both sites are shown on **Figure 5-200**.

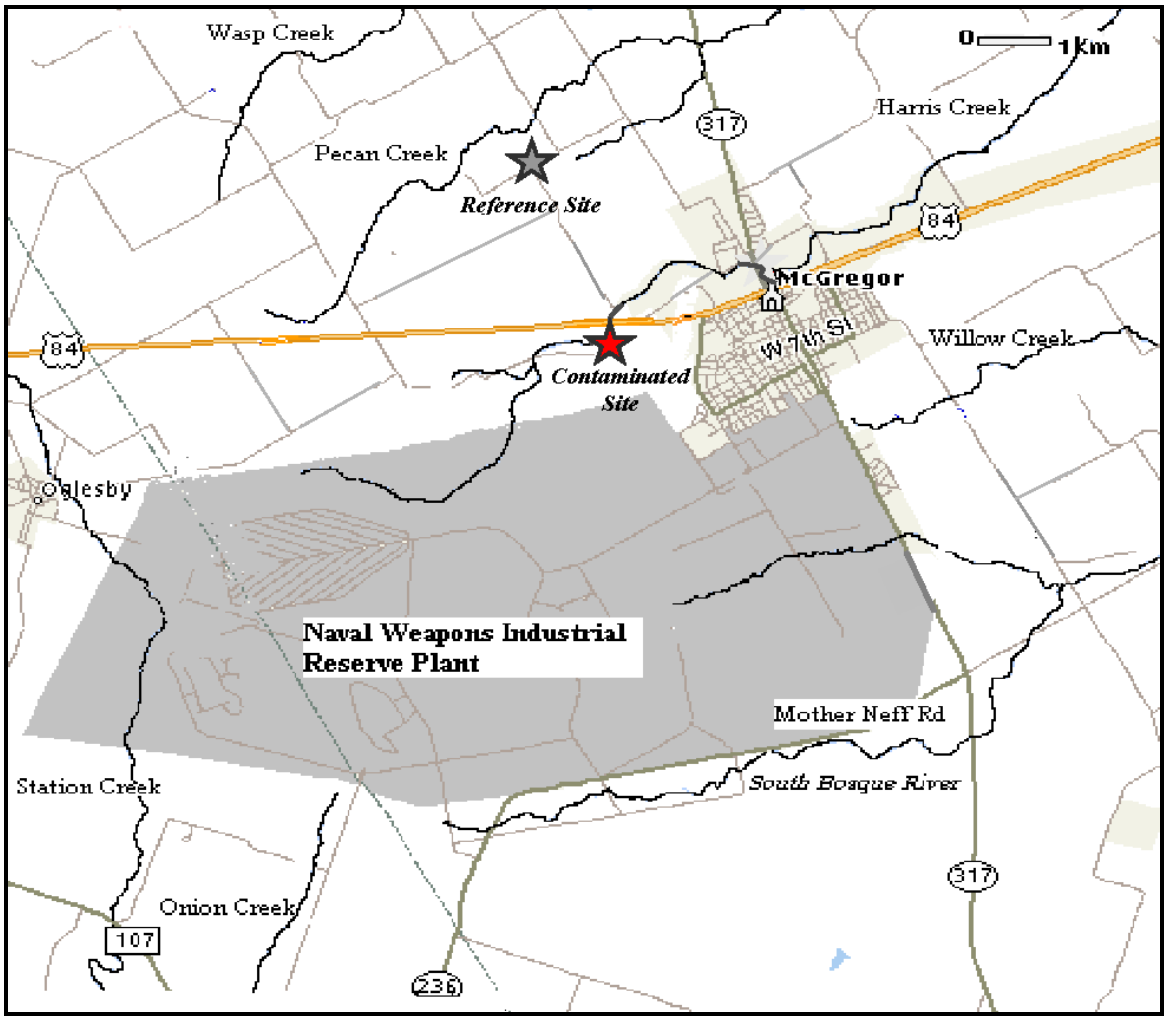

Figure 5-200
Locations of the Reference Site and the Contaminated Site for the Cattle Study

Heifers on both sites were visually monitored on a daily basis and blood was collected from each animal every 2 weeks. Blood samples were always collected in the morning (before 9:00 am). At the time of blood collection, drinking water that the animals had access to was also collected. Plasma and serum from the blood were isolated in the field via centrifugation. Samples were stored on dry ice during transport back to the laboratory, and stored frozen (-80 °C) until analysis.

At the conclusion of the study, the heifers described above and two older cows that had resided on the contaminated property for several years, were processed in a manner identical to a commercial beef processing operation. The following tissue samples were obtained from each heifer for residue analysis: liver and various meat cuts (sirloin steak, round steak, t-bone steak, and roast). Liver, ground beef, and "chili meat" from the two older cows were also examined for perchlorate residues.

5.5.3.1.1.2 Sample Preparation and Perchlorate Analyses

A potassium perchlorate ($KClO_4$) standard solution was obtained as a custom standard from AccuStandard, Inc. (New Haven, CT). Sodium hydroxide (NaOH), 50% (w/w) aqueous solution was purchased from Fisher Scientific. All solutions were prepared in 18.2 MΩ Milli-Q water. Ethanol was purchased from Fisher Scientific.

Plasma samples were processed using methods similar to those described previously (Fisher et al., 2000; Anderson and Wu, 2002; Narayanan et al., 2003) prior to analysis. First, 1 mL of plasma was precipitated with 4 mL of ethanol (ice-cold) and then centrifuged (4 °C) at 3750 rpm for 5 min. The supernatant was removed, evaporated to dryness under nitrogen, and reconstituted in 5 mL Milli-Q water (Fisher et al., 2000; Narayanan et al., 2003). Samples were then cleaned up using Alumina and C_{18} solid phase extraction (SPE) cartridges, and filtered (0.45 μm) prior to IC analysis.

Tissue samples were also processed prior to IC analysis. Samples (10- 20 g) were air dried and then extracted with Milli-Q water using Accelerated Solvent Extraction (ASE; Dionex Corp.) Extraction conditions were as follows: pressure = 1500 psi, temperature = 100 ºC, extraction time = 15 min. Sample extract volumes were measured, diluted (5x), and cleaned up with Alumina and C_{18} SPE cartridges. Eluates were filtered (0.45 μm) prior to IC analysis.

Surface water samples collected from the two sites during the course of the study were filtered (0.45 μm) prior to analysis.

All samples (plasma extracts, tissue extracts, and surface water) were analyzed by ion chromatography similar to EPA Method 314 (See **Appendix X** for specific procedures).

5.5.3.1.2 Data

Perchlorate was not detected in water samples collected from the reference site. In contrast, perchlorate was detected throughout the study in water samples from the treatment site (25.4 ± 5.8 ng/mL; n = 8). The levels of perchlorate in water from the treatment site are consistent with more than two years of monitoring data from that location.

Perchlorate was not detected in blood plasma samples from either cow occupying the reference site (**Figure 5-201**). Similar results were obtained for the two cows on the treatment site with one exception; perchlorate was detected (15 ng/mL and 22 ng/mL) in one of the cows on consecutive sampling periods four and six weeks after the beginning of perchlorate exposure.

Perchlorate was not detected in any of the tissue samples collected from heifers occupying the reference site. In addition, perchlorate was not detected in any of the tissue samples collected from heifer cows occupying the treatment site.

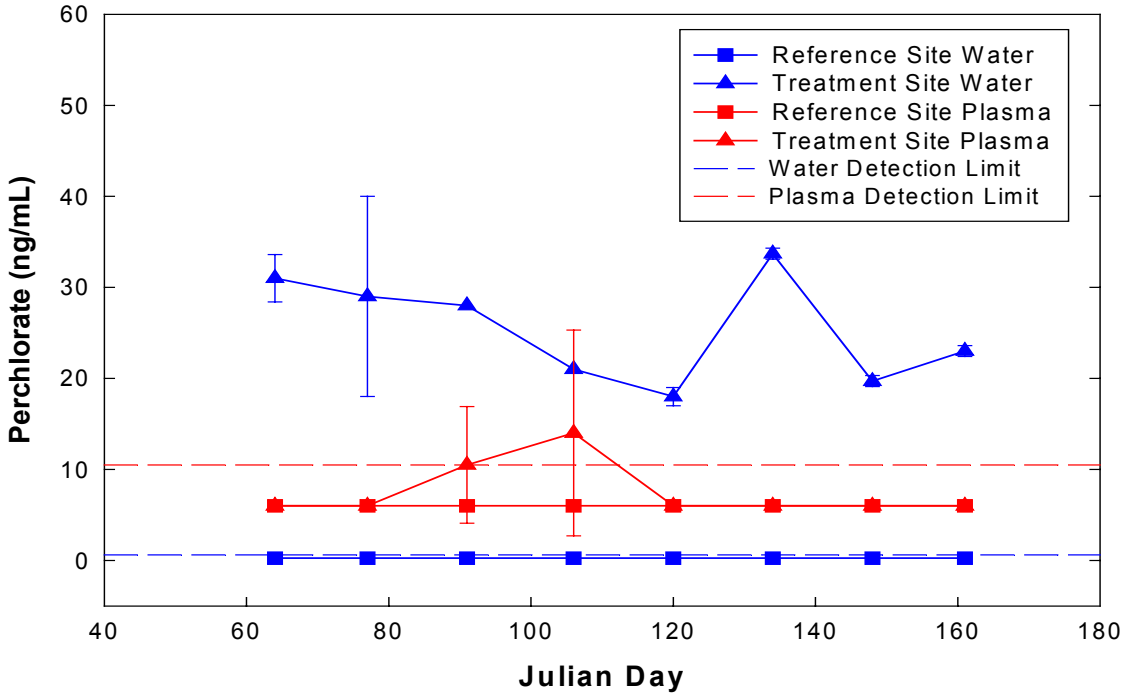

Figure 5-201
The Relationship Between Perchlorate Exposure in Drinking Water and Perchlorate in Cow Plasma

All reference cattle plasma data were below the analytical limit of detection (11 ppb) and were therefore assigned a value of one half the detection (limit 5.5 ppb) for data presentation. Similarly, all reference water data were below detection limit and are presented as one half the detection limit (0.5 ppb).

5.5.3.1.3 Discussion

Our results indicate that despite the presence of perchlorate in drinking water at the treatment site, there was little quantifiable exposure measured in blood plasma from the cattle. In other words, constant exposure to 25 ppb perchlorate in water over 14 weeks did not result in measurable residues in blood plasma. Water intake in cattle is reasonably well understood. An adult cow takes in around 40 L of water per day and excretes around 30 L per day (20 L in feces, 10 L in urine). Based on this assumption and the measured drinking water concentrations, the cows on the Treatment Site were ingesting 250 µg perchlorate per day. If one makes the typical assumption that a cow has 60 mL of blood per kg body weight, the expected perchlorate concentration in plasma should be around 18 ppb. This value is consistent with the 2 detections that were observed (15 ppb and 22 ppb).

Perchlorate was detected with greater frequency and at higher concentrations in cattle from two Kansas farms adjacent to facilities that used or handled perchlorate. Cattle on these farms were not restricted to water supplies containing perchlorate as were those on the McGregor treatment site. Nonetheless, perchlorate was detected in 4/33 and 17/26

cattle at the two Kansas farms. The highest plasma perchlorate concentrations observed in the Kansas cattle were 43 and 32 ppb, respectively.

Perchlorate can be rapidly excreted in urine, with reported urinary excretion half-lives ranging from 8 to 20 hours in rats (Wolff, 1998; Fisher et al., 2000). Perchlorate accumulation was not consistently observed in plasma from cows on the McGregor treatment site, most likely due to rapid excretion rates. Batjoens et al. (1993) reported that prolonged perchlorate administration (4 g/day for 10 days) in cows resulted in a relatively longer excretion period in urine which may have been more indicative of exposure than plasma as was examined in our study.

Considerable effort was made to develop a method for perchlorate analysis in plasma (See **Appendix X**). The method can reduce the typical background interference significantly, and perchlorate recovery of spiked samples is consistent and reproducible (85.3 ± 0.5%). In addition, the detection limit for perchlorate in blood plasma (S/N = 3) was low enough (11 ppb) to detect perchlorate if present.

5.5.3.2 Thyroid Hormones in Large Mammals

5.5.3.2.1 Methodology

Plasma was analyzed for total T_3 and total T_4 using Coat-A-Count radioimmunoassay (RIA) kits (Diagnostic Products Corporation DPC, Los Angeles, CA). The assay was performed according to manufacturer's instructions. Plasma samples were assayed on a Packard Cobra E5005 gamma counter. Samples were assayed in triplicate and standards (six points) that came with the kit were run at the beginning of each assay and intermittently throughout the assay.

5.5.3.2.2 Data

Plasma samples were collected from individual heifers approximately every two weeks from March 5, 2003 to June 10, 2003. Individual heifer thyroid hormone concentrations varied considerably from week to week (**Figure 5-202** and **Figure 5-204**). T_3 concentrations ranged from 0.83 to 1.75 ng/mL with a mean concentration of 1.26 ± 0.07 ng/mL standard error (SE) in the perchlorate-exposed heifers. Reference heifer T_3 concentrations ranged from 1.24 to 2.11 ng/mL with a mean concentration of 1.61 ± 0.07 ng/ml SE. Mean T_3 concentrations ranked from highest to lowest were: 152 control = 1.73 ± 0.08 ng/mL, 153 control = 1.50 ± 0.11 ng/mL, 154 treated = 1.36 ± 0.09 ng/mL, and 151 treated = 1.16 ± 0.11 ng/mL.

T_4 concentrations ranged from 39.47 to 80.80 ng/mL with a mean concentration of 55.58 ± 3.36 ng/ml SE in the perchlorate-exposed heifers. Reference heifer T_4 concentrations ranged from 41.38 to 88.19 ng/mL with a mean concentration of 64.38 ± 3.38 ng/mL SE. Mean T_4 concentrations ranked from highest to lowest were: 152 control = 70.73 ± 2.57 ng/mL SE, 151 treated = 65.80 ± 3.72 ng/mL, 153 control = 58.03 ± 5.55 ng/mL SE, and 154 treated = 45.37 ± 2.15 ng/mL. There was a noticeable decrease in T_4 concentrations among all cattle on the April 16, 2003 sampling period (**Figure 5-204**) that may have been due to assay-related error. Overall, there appeared to be a general trend of

decreasing thyroid hormones in both the reference and the exposed animals (**Figure 5-203** and **Figure 5-205**).

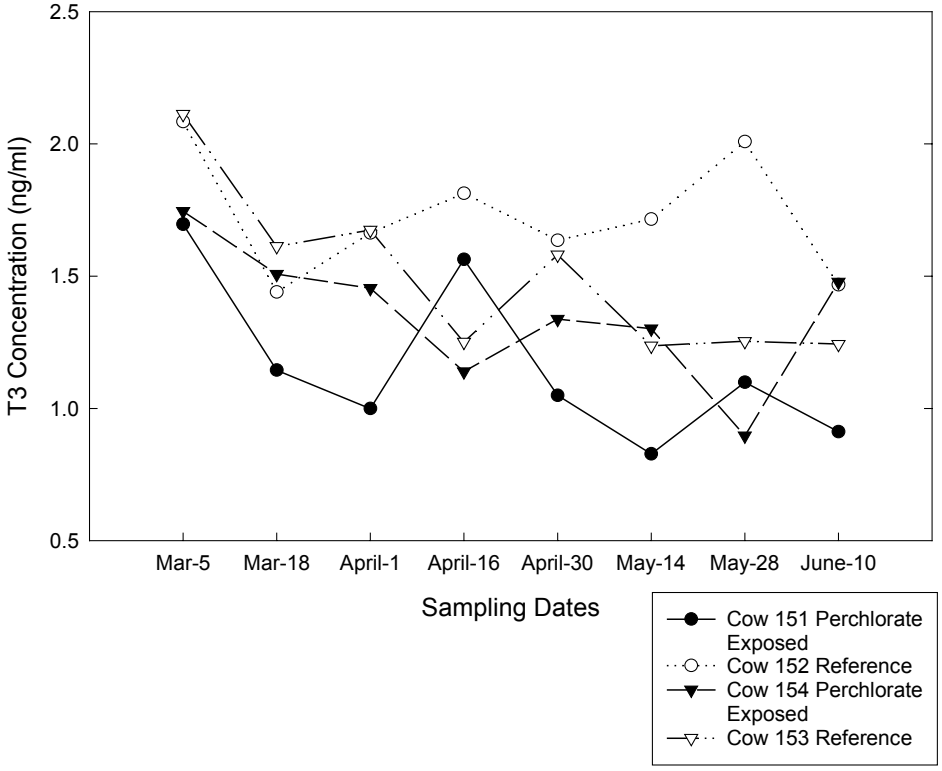

Figure 5-202
Total T_3 Concentrations in Plasma from Four Heifers on Either Perchlorate-Contaminated (151, 154) or Reference Areas (152, 153) near McGregor, TX 2003

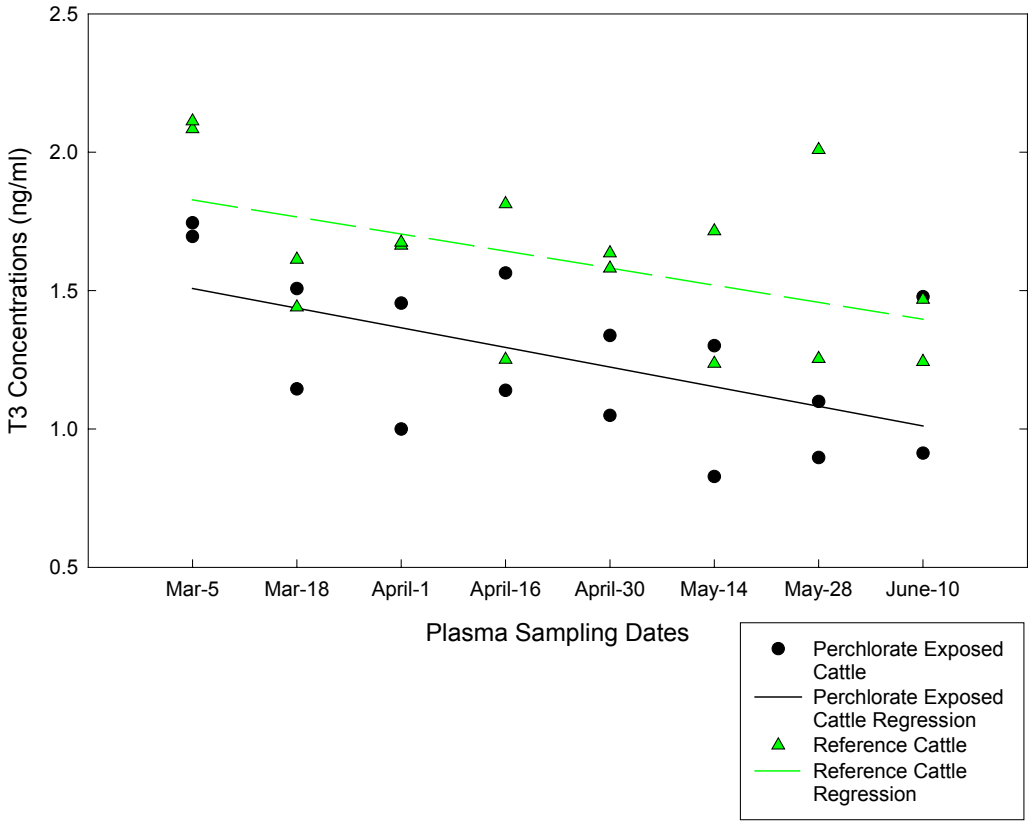

Figure 5-203
Linear Regression of Plasma T_3 Concentrations in Heifers Maintained on a Perchlorate-Contaminated, or a Reference Pasture near McGregor, TX, 2003

(The slope for the reference heifer regression line is -0.06 and the r^2 is 0.25. The slope for the perchlorate-exposed heifer regression line is -0.07 and the r^2 is 0.33.)

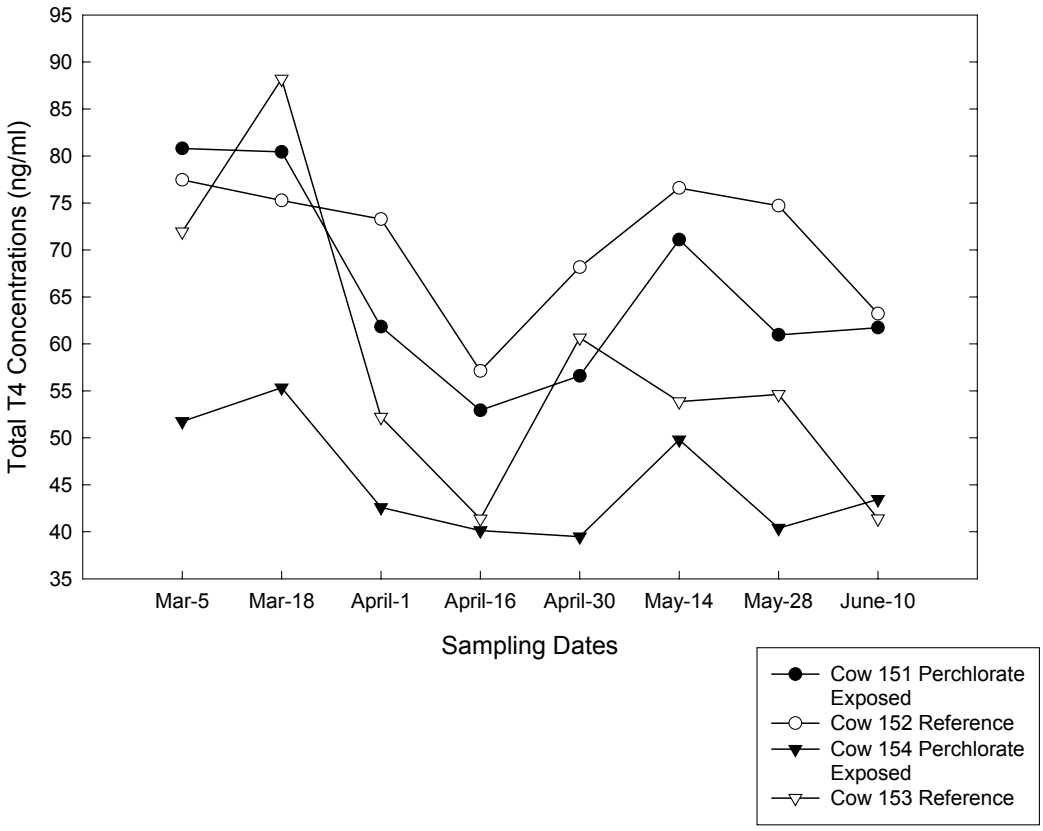

Figure 5-204
Total T_4 Concentrations in Plasma from Four Heifers on Either Perchlorate-Contaminated (151, 154) or Reference Pastures (152, 153) near McGregor, TX 2003

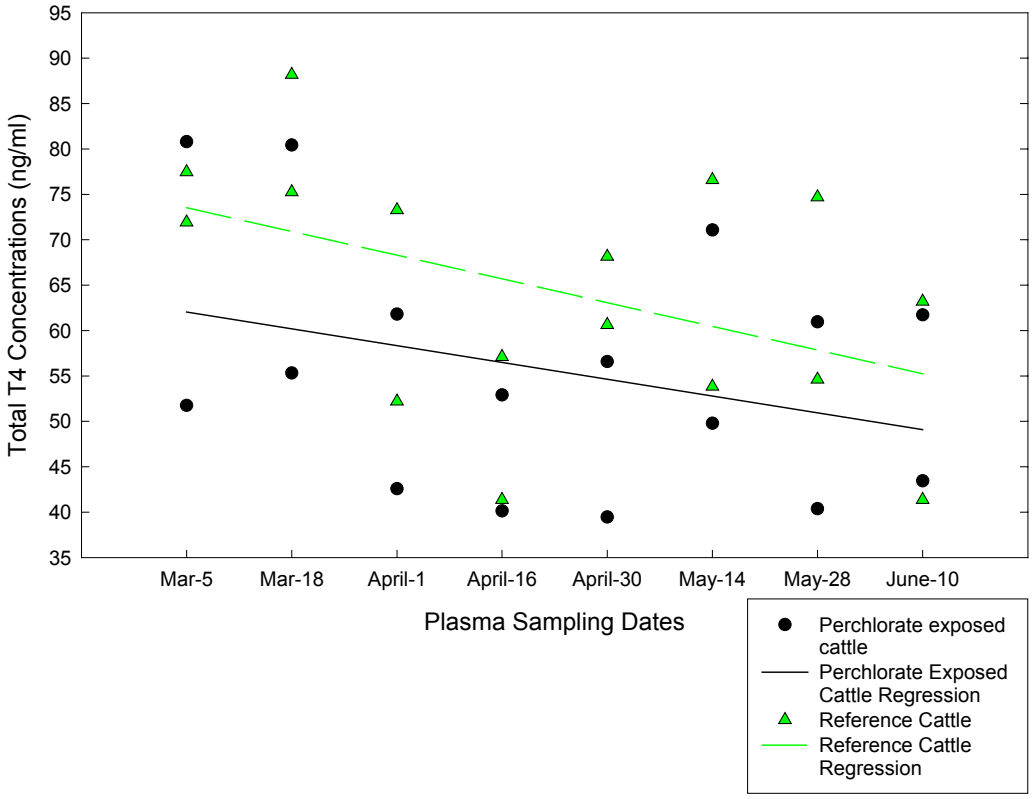

Figure 5-205
Linear Regression of T_4 Plasma Concentrations in Heifers Maintained on a Perchlorate-Contaminated or a Reference Pasture near McGregor, TX, 2003

(The slope for the reference heifer regression line is -2.61 and the r^2 is 0.21. The slope for the perchlorate-exposed heifer regression line is -1.85 and the r^2 is 0.11.)

5.5.3.2.3 Discussion

There was considerable variability in thyroid hormone concentrations in both perchlorate-exposed and reference heifers, and there did not appear to be any perchlorate-related reductions in thyroid hormone concentrations. Perchlorate is known to cause hypothyroidism; characterized by a decrease in thyroid hormone levels, an increase in weight and lethargy (Mannisto et al., 1979). De Moraes et al. (1998) reported average blood serum concentrations for T_3 at 1.6 ng/mL and for T_4 at 88.5 ng/mL collected over a five week period from Brahman cattle. Grigsby and Trenkle (1986) reported average blood plasma T3 concentrations of 1.75, 1.22, 1.52 ng/mL and for T_4 concentrations of 72.5, 70.5, 81.0 ng/mL in Angus, Limousin, and Simmental steers, respectively. These studies illustrate the variability in thyroid hormone concentrations among cattle breeds. Our results are comparable to those of Grigsby and Trenkle (1986). The cattle used for this study were mixed breed which may have contributed to the variability observed in thyroid hormone concentrations. The perchlorate-exposed cattle mean thyroid hormone concentrations were lower than those of the reference heifers throughout the study, and

the rate of decrease in thyroid hormones throughout the study periods were similar between the two treatment groups (**Figure 5-203** and **Figure 5-205**). In fact, the rate of decline in T_4 concentrations among the reference heifers was slightly greater than that of the perchlorate-exposed heifers. Kansas cattle potentially exposed to perchlorate exhibited a negative relationship between thyroid hormone concentrations and age, but no noticeable alterations in thyroid hormone status that could be linked to perchlorate exposure (TIEHH data on file). Therefore, it is doubtful that perchlorate inhibited thyroid hormone production in the McGregor treatment site (or reference) heifers. Therefore, we conclude that low concentrations of perchlorate in forage and water do not significantly affect sub-adult cattle thyroid hormone concentrations. However, additional studies are needed to assess developmental effects in perchlorate-exposed calves, and the effects of longer-term exposures.